Kit Preparation of Radioiodinated *o*-Iodohippuran

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Abstract □ The purpose of this study was to evaluate a kit preparation for radioiodinated *o*-iodohippuran (I). All ingredients, excluding the radionuclide, were packaged in a ready-to-use kit for easy, quick formulation. Electrophoresis was utilized to evaluate the radiochemical purity of the labeled product and indicated that the radiolabeling technique provided a product with >95% radiochemical purity. Biodistribution studies in rats and rabbits provided an indication of the tissue distribution and localization of the radiopharmaceutical. Computer-generated renogram curves plotted from γ-camera images of rabbits showed the equivalency of the ^{131}I-labeled I and ^{123}I-labeled I to the commercially available radiopharmaceutical.

Keyphrases □ *o*-Iodohippuran, radioiodinated—kit preparation evaluated and compared with commercial products, biodistribution studies in rats and rabbits □ Radiopharmaceuticals—*o*-iodohippuran labeled with iodine 123 and with iodine 131, kit preparation method developed and compared with commercial products, biodistribution studies in rats and rabbits □ Biodistribution—radioiodinated *o*-iodohippuran in rats and rabbits, kit preparation of radiopharmaceutical developed and compared with commercial products

o-Iodohippurate sodium (I) radiolabeled with iodine 131 is used extensively for the noninvasive assessment of kidney function. Diagnostic information regarding renal blood flow, urinary tract patency, and urine flow may be obtained quickly and easily.

The clinical significance of a renal study with radioiodinated I may be improved by using iodine 123 rather than iodine 131 (1–5). The short physical half-life (13 hr) of iodine 123 and its pure γ-photon emission decrease the absorbed radiation dose per millicurie to the patient compared to iodine 131 (6). In addition, iodine 123 produces improved images with nuclear medicine instrumentation compared to iodine 131. The γ-photons emitted from iodine 131 are too energetic to be detected efficiently by the instrumentation generally used today. However, the 0.159 Mev γ-photons emitted from iodine 123 are detected more efficiently using a γ-camera and thus provide better counting statistics.

The relatively short physical half-life is a disadvantage in the use of iodine 123. The radiopharmaceutical, *i.e.*, ^{123}I-labeled I, must be prepared at the institution where it will be used to make the agent economically feasible. Thus, the preparation method must be in the form of a radiopharmaceutical kit similar to kits used to prepare ^{99m}Tc-labeled radiopharmaceuticals. Previous means for producing radioiodinated I (4, 7–15) involved extensive radiochemical synthetic and purification methods that do not allow the agent to be prepared easily at the site of use.

A rapid method for exchanging iodine 125 with the nonradioactive iodine in I was presented recently (16). The objectives of this research were to modify that method (16) to produce ^{123}I-labeled I in kit form and to evaluate its biological distribution in animals.

EXPERIMENTAL

Materials—*o*-Iodohippuric acid[1] was extracted three times with benzene (17) to remove traces of *o*-iodobenzoic acid (II), which competes with I for radioiodine during the exchange reaction (16). Melting-point determination and high-pressure liquid chromatographic (HPLC) analysis indicated that extraction effectively removed traces of II from I.

A solution of 50 mg of anhydrous copper sulfate in 10 ml of distilled water further enhanced the labeling of I by preferentially binding to any remaining II. Phosphate buffer was prepared by dissolving 6.185 g of dibasic sodium phosphate in 100 ml of distilled water.

Sodium [^{131}I]iodide was obtained commercially[2]. The 0.1 *N* NaOH solution of the sodium [^{123}I]iodide[3] was reduced to pH 3–7 with 0.5 *N* HCl prior to radioiodination of I.

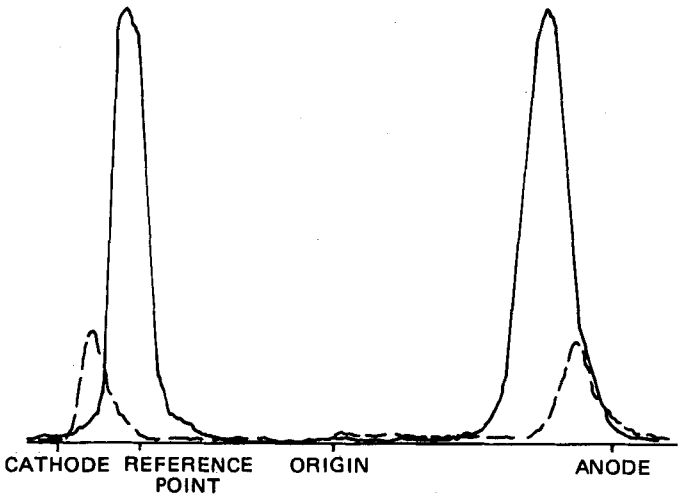

Figure 1—*Radiochromatogram scans of electrophoresis strips of sodium [^{131}I]iodide (—) and sodium [^{123}I]iodide (– –) showing the movement of radioiodine in this form toward the anode.*

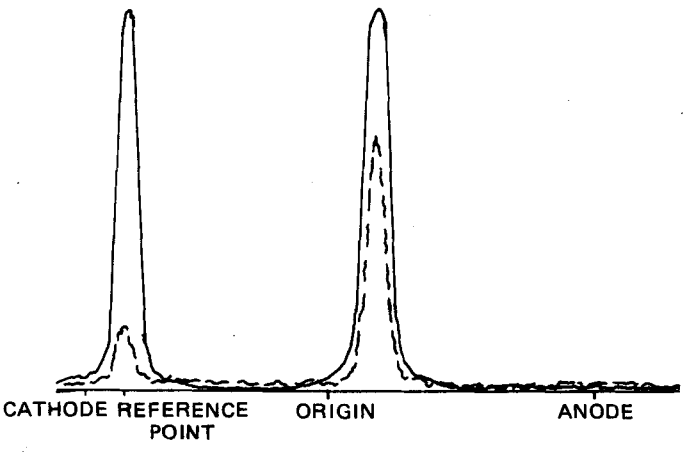

Figure 2—*Radiochromatogram scans of electrophoresis strips of ^{131}I-labeled I (—) and ^{123}I-labeled I (– –) showing the movement of the labeled compound.*

[1] Aldrich Chemical Co., Milwaukee, Wis.
[2] Mallinckrodt Nuclear, St. Louis., Mo., and E. R. Squibb & Sons, Princeton, N.J.
[3] Crocker Nuclear Laboratory, Davis, CA 95616.

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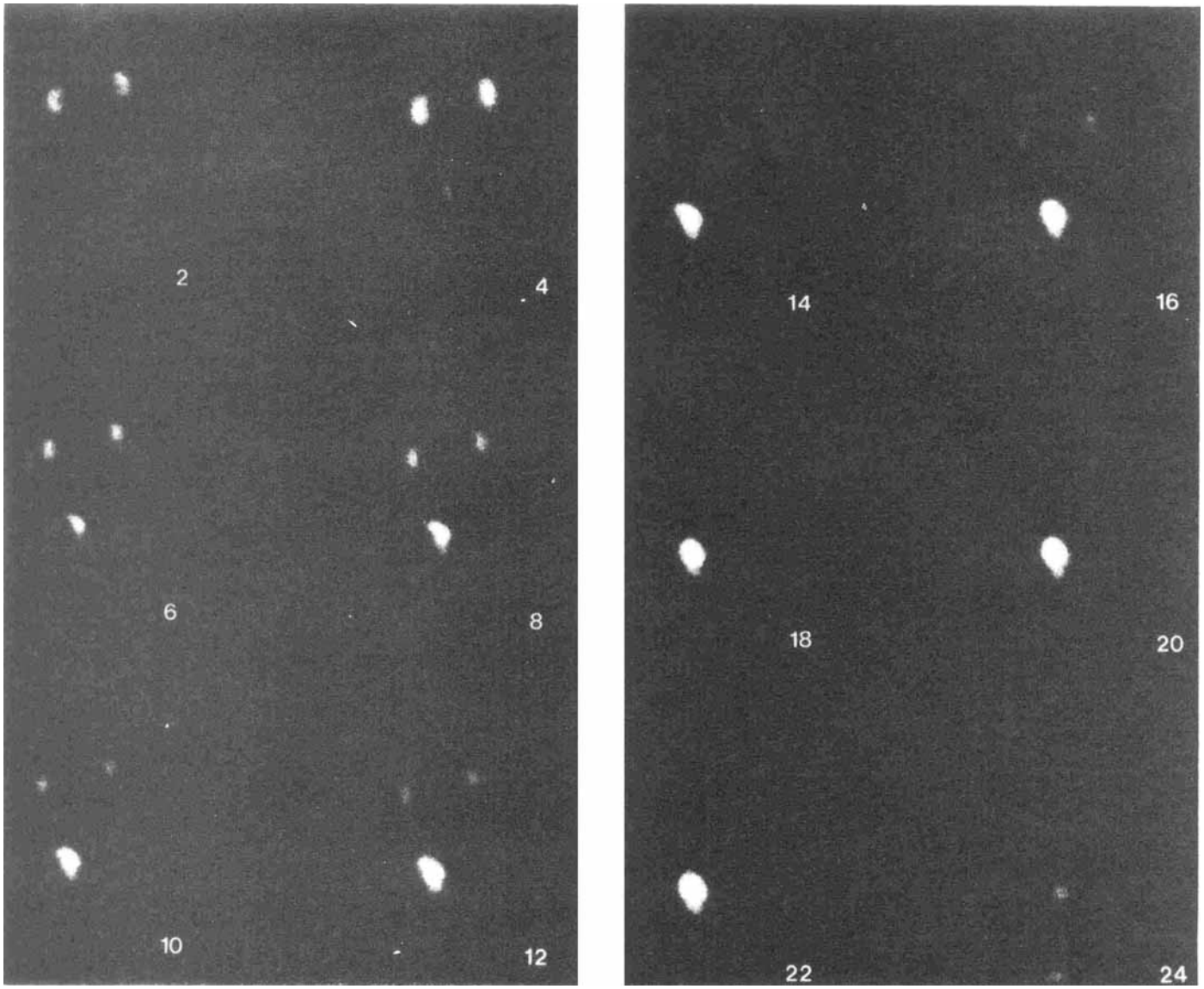

Figure 3—*γ-Camera image of rabbit biodistribution of kit-prepared ^{123}I-labeled I. Each frame represents 2 min of elapsed time.*

Lyophilization—To prepare 10 lyophilized vials, 50 mg of purified I was dissolved in 1 ml of absolute ethanol, and 6 ml of distilled water was added. One milliliter of the copper sulfate–distilled water solution (5 mg/ml) was added to bring the final volume to 10 ml. Aliquots (1 ml) were filtered through a 0.22-μm filter[4] into 10-ml serum vials. The solutions were frozen rapidly with solid carbon dioxide lyophilized[5] for 24 hr, and the vials were stoppered and sealed. Each vial contained 5 mg of I and 0.5 mg of copper sulfate in lyophilized form.

Preparation of Radioiodinated I—From 0.5 to 5.0 mCi of radioiodine (either sodium [^{123}I]iodide or sodium [^{131}I]iodide) was added to the vial containing the lyophilized I and copper sulfate. The vial was autoclaved[6] for 15 min at 120–130° and allowed to cool at room temperature. Phosphate buffer (2 ml) was added to precipitate the copper as copper phosphate and to raise the pH to 7–8. Then the solution was passed through a 0.22-μm filter[4] into a clean 10-ml evacuated serum vial. The total activity was measured in a dose calibrator[7], and the specific concentration was calculated.

Radiochemical Purity—A sample of radioiodinated I was placed on the center of a cellulose polyacetate electrophoresis strip[8]. A similar sample of radioiodinated sodium iodide was placed on a second strip as a control. In addition, a third strip was spotted with a small amount of radioiodinated II. The strips were placed in an electrophoresis chamber[8] containing tromethamine–barbital–barbital sodium buffer[8] (pH 8.8, ionic strength 0.06). The voltage was set at 275 v and allowed to run for 15 min. Then the strips were removed from the chamber and allowed to air dry. A small amount of radioactive material was placed on the cathode end of each strip as a reference point, and the position of radioactivity on each strip was determined with a radiochromatogram scanner[9].

Biodistribution—Five millicuries of ^{123}I-labeled I was prepared as described, and the radiochemical purity was determined by electrophoresis.

Normal male Sprague–Dawley rats, 300–400 g, were weighed and anesthetized with chloroform. A dose (0.1 ml) of ^{123}I-labeled I then was administered intravenously into the femoral vein following a femoral vein cutdown procedure.

Two rats were sacrificed at 1, 2, 5, 10, and 30 min following injection. Duplicate samples of blood, liver, heart, small intestines, kidneys, fat, and testes were removed from each rat, weighed, and placed in counting tubes.

A standard for counting was prepared by diluting 0.1 ml of the preparation to 100 ml with saline. Each tube, except the tubes containing kidney samples, were counted in an automatic γ-counter[10] for 1 min. A 0.1-ml aliquot of the standard was placed before and after each set of

[4] Millipore.
[5] Labconco Freeze-Dry, Labconco Corp., Kansas City, Mo.
[6] Model 1250 Labclave, Ritter Sybron Corp., Rochester, N.Y.
[7] Model CRC-10R radioisotope calibrator, Capintec Inc., Mount Vernon, N.Y.
[8] Gelman, Ann Arbor, Mich.
[9] Model 930, Vangard Systems, Stamford, Conn.
[10] Gamma 300, Beckman Instruments, Fullerton, Calif.

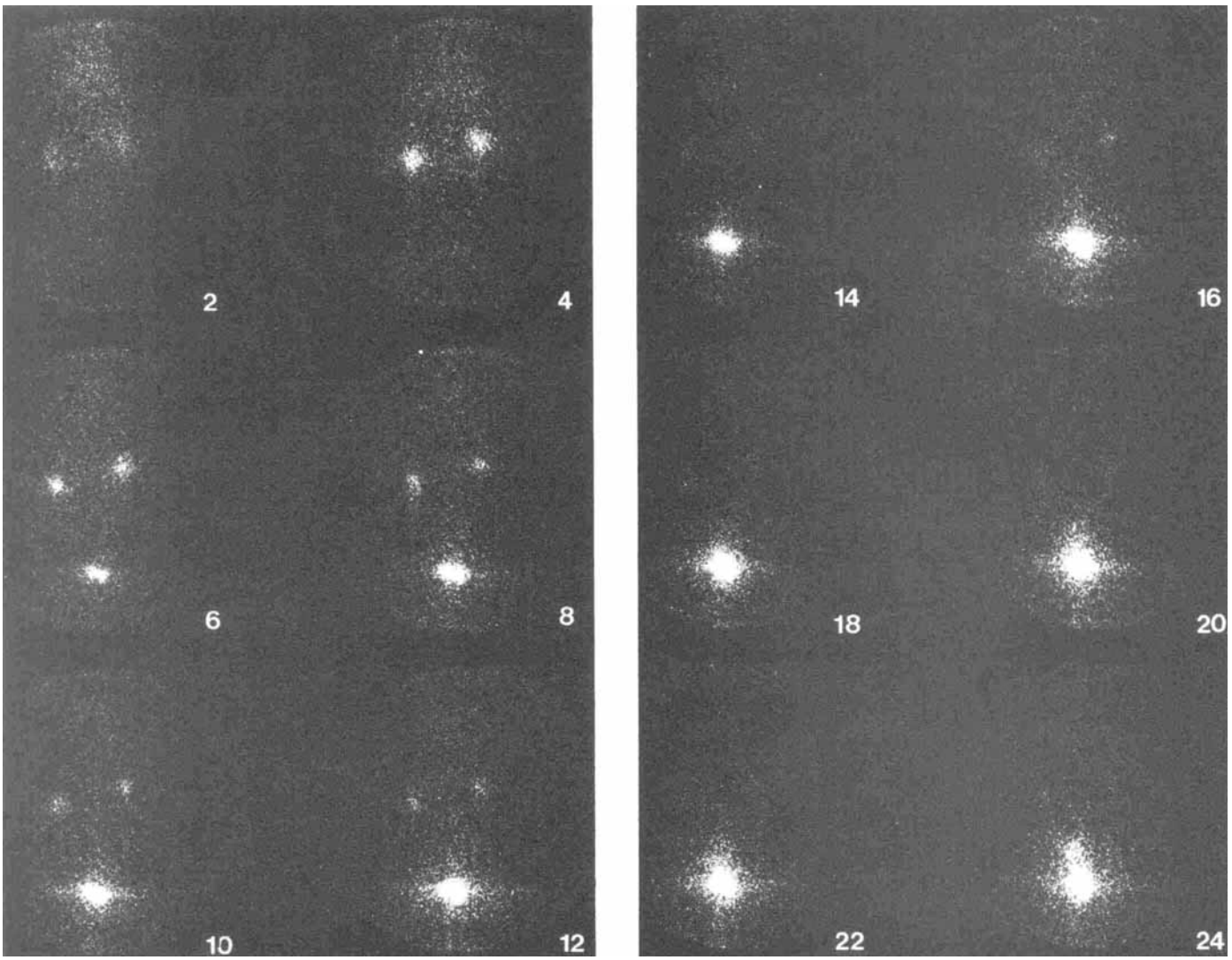

Figure 4—*γ-Camera image of rabbit biodistribution of kit-prepared ^{131}I-labeled I. Each frame represents 2 min of elapsed time.*

tissue samples from a given rat. Kidney samples were counted ~39 hr after the other samples because of the extremely high levels of radioactivity, which would have caused an error in the results due to resolving time losses.

The tissue levels of radioactivity are described as percent of kilogram dose per gram (% kg dose/g) (18):

$$\%\ \text{kg dose/g} = \frac{\text{cpm in tissue/g of tissue}}{\text{cpm in dose/kg wt. of animal}} \times 100\% \quad \text{(Eq. 1)}$$

where cpm in dose = cpm in diluted dose × 1000.

Rabbit Studies—White, female, New Zealand rabbits were anesthetized using a combination of xylazine[11] (10 mg/kg) and ketamine[12] (35 mg/kg) injected intramuscularly. The rabbits were injected intravenously with 0.1–0.5 mCi of ^{123}I-labeled I prepared by the kit method or ^{131}I-labeled I obtained commercially[13] or prepared from a kit. The γ-camera[14] images were taken 2 min apart for 30 min. Renogram curves were obtained through the use of a computer interphased with the γ-camera.

RESULTS

Radiochemical Purity—The pH of the radioiodine solution used in preparing the radiopharmaceutical was found to be important. Commercially available sodium [^{131}I]iodide solutions have pH values of 3–7, which is acceptable. However, the sodium [^{123}I]iodide utilized in this study was supplied in 0.1 *N* NaOH. The pH of this radioiodine solution must be ≤7 to label I efficiently using the kit described.

Electrophoresis was an efficient method for evaluating the radiochemical purity of radioiodinated I. Figure 1 illustrates the expected migration of $^{131}I^-$ and $^{123}I^-$ toward the anode. Both ^{131}I-labeled I and ^{123}I-labeled I also traveled toward the anode (Fig. 2) with an R_I- of ~0.3–0.4, identical to that of commercially available ^{131}I-labeled I. Radioiodinated II traveled toward the anode with an R_I- of 0.17. This finding illustrates the system's ability to separate between the radioiodinated I and the radioiodinated II impurity. Greater than 95% radiochemical purity was achieved utilizing the kit method in the preparation of these agents.

Electrophoresis of the two radiolabeled compounds at various times after preparation indicated that the radiopharmaceutical was stable for at least 10 days when stored at room temperature.

Biodistribution—The ^{123}I-labeled I prepared by the kit method (Table I) was cleared rapidly from the blood. Kidney concentration of radioactivity reached a maximum between 1 and 5 min after injection and then declined rapidly. Radioactivity in organs other than the kidneys was low relative to the kidneys. Free radioiodine localizes to a large extent in the intestine (19). The relatively low concentration of radioactivity in the small intestine indicated good *in vivo* stability of ^{123}I-labeled I.

Rabbit Studies—Of major concern in the development of a new method of preparing a radiopharmaceutical that is available commercially is that the nuclear medicine images obtained are similar or improved. To evaluate this point, rabbits were injected with commercially available

[11] Chemagro, Kansas City, Mo.
[12] Bristol Laboratories, Syracuse, N.Y.
[13] Mallinckrodt Nuclear, St. Louis, Mo.
[14] LFOV, Searle, Chicago, Ill.

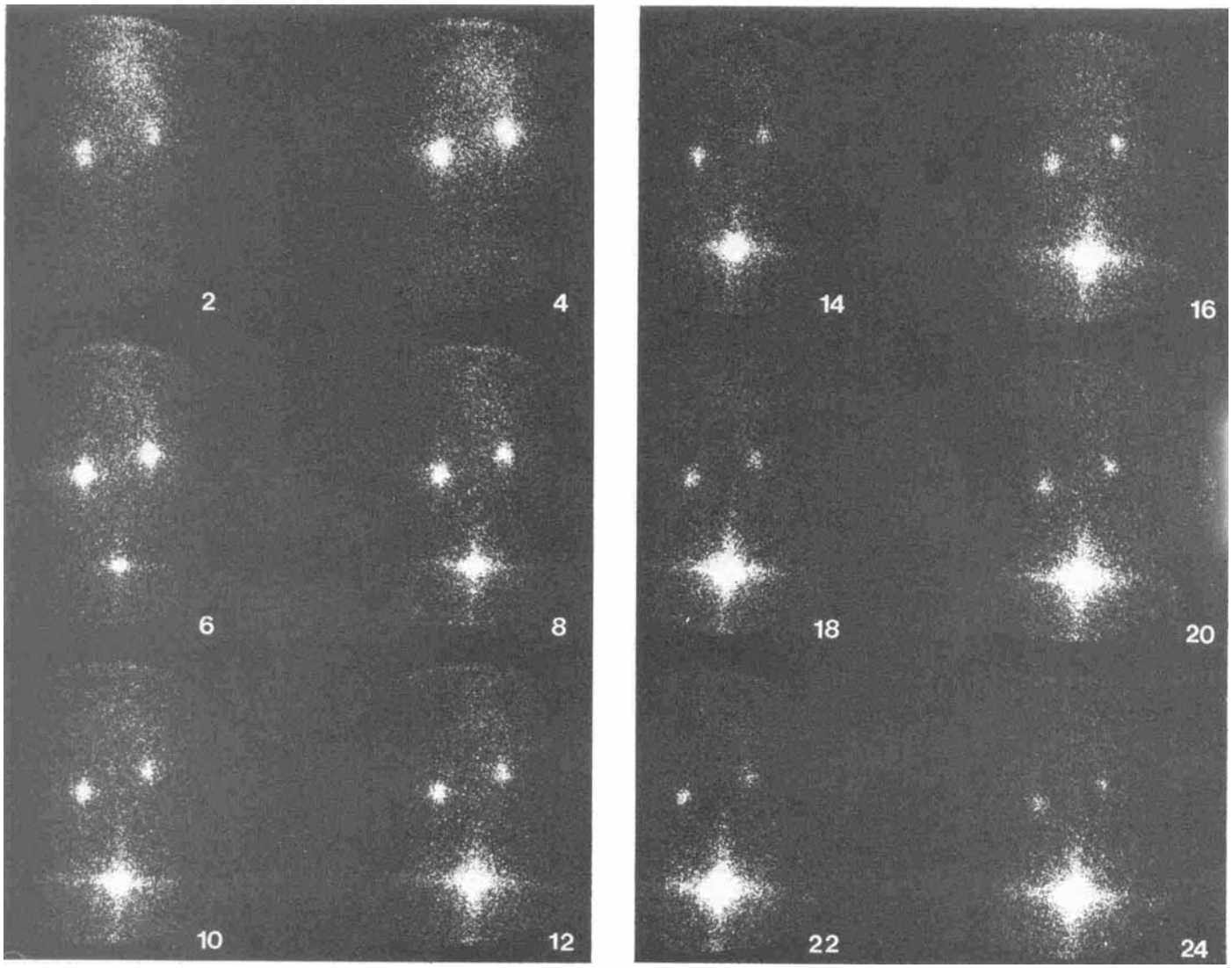

Figure 5—*γ-Camera image of rabbit biodistribution of commercially available ^{131}I-labeled I. Each frame represents 2 min of elapsed time.*

^{131}I-labeled I and kit-prepared ^{131}I-labeled I and ^{123}I-labeled I. The images obtained using standard nuclear medicine instrumentation and procedures were comparable with all three preparations (Figs. 3–5).

Kidneys were seen within the first 2 min after injection. Urinary bladder was visible within 4–6 min following injection. At 20 min, all of the radioactivity appeared to have localized in the urinary bladder with faint shadows of the kidneys visible. The nonvisualization of the thyroid supports the conclusion that an *in vivo* stable product is formed. The images obtained with ^{123}I-labeled I (Fig. 3) were superior to those obtained with ^{131}I-labeled I (Fig. 4) prepared by the kit method and ^{131}I-labeled I (Fig. 5) obtained commercially. This improved imaging was due to the higher photon flux and the more efficiently detected γ-photon of iodine 123. Renograms (Fig. 6) generated by computer accumulation of the distribution of radioactivity also were comparable with all three preparations.

DISCUSSION

The kit method for preparing ^{123}I-labeled I and ^{131}I-labeled I yields a radiochemically pure agent. The method is quick and easy, with the final product obtained 60 min after the addition of radioiodine to the reaction vial.

The radiopharmaceutical kit consists of: (*a*) a 10-ml sterile, pyrogen-free, evacuated vial containing 5 mg of purified I and 0.5 mg of copper sulfate in lyophilized form; (*b*) a 3-ml disposable glass syringe containing 2 ml of dibasic sodium phosphate buffer (6.185 g/100 ml); (*c*) a 0.22-μm sterile disposable filter; (*d*) a 10-ml sterile, pyrogen-free, evacuated serum vial; and (*e*) a 5-ml syringe.

The γ-camera images of rabbits and computer-generated renograms following intravenous injection of the kit preparation were of excellent quality and comparable to those following intravenous injection of

Table I—Tissue Distribution [a] of [^{123}I]-*o*-Iodohippuran in Rats

Minutes after Injection	Blood	Kidneys	Liver	Heart	Small Intestine	Testes	Fat
1	0.650	2.69	0.330	0.187	0.092	0.041	0.020
2	0.508	2.75	0.337	0.191	0.141	0.036	0.036
5	0.188	1.56	0.130	0.064	0.048	0.033	0.031
10	0.109	0.822	0.067	0.032	0.027	0.019	0.012
30	0.046	0.170	0.020	0.010	0.036	0.009	0.010

[a] Mean percent of kilogram dose per gram.

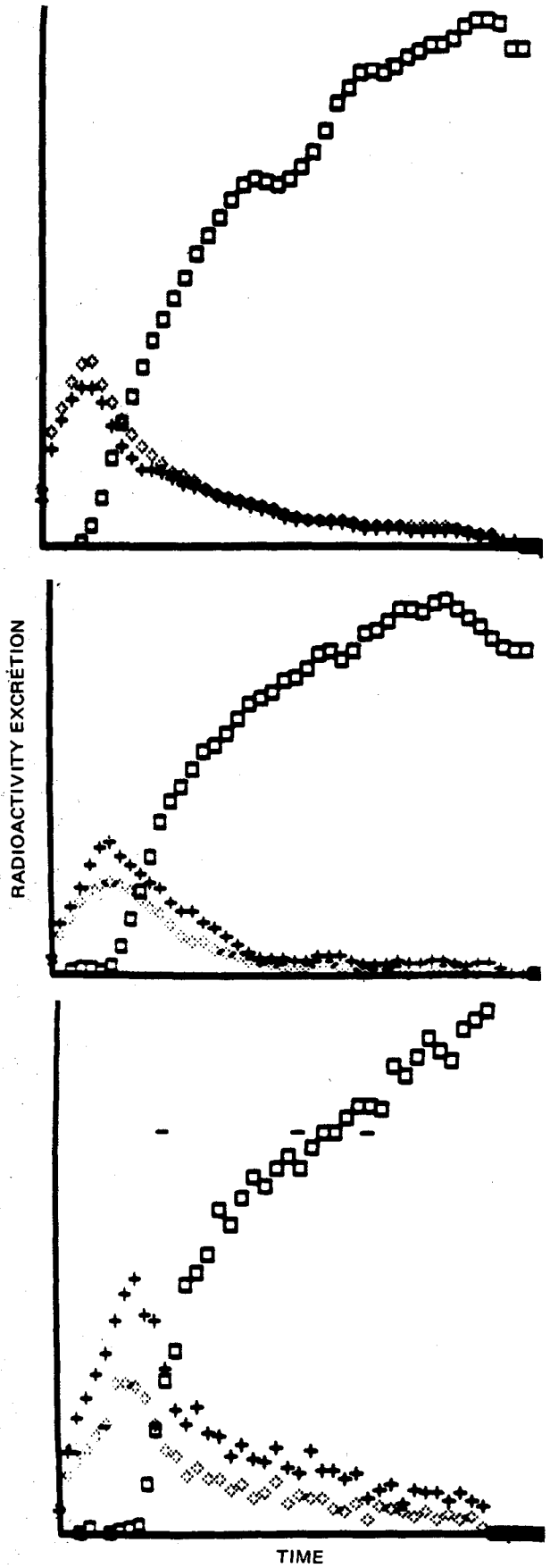

Figure 6—*Renogram curves showing the excretion of ^{123}I-labeled I prepared by the kit method (top), ^{131}I-labeled I prepared by the kit method (middle), and ^{131}I-labeled I obtained commercially (bottom). Each point on a line represents 30 sec of elapsed time. The squares represent the radioactivity in the bladder while the crosses and diamonds represent the radioactivity in each kidney.*

commercially obtained ^{131}I-labeled I.

This method for preparing radioiodinated I, especially ^{123}I-labeled I, will be evaluated for human use. It would allow nuclear pharmacies and nuclear medicine units to prepare ^{123}I-labeled I at the site of use and thus reduce the cost of the agent, reduce the radiation dose to the patient, and improve the clinical evaluation.

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