Preparation and Use of I^{131} Labeled Sodium Iodohippurate in Kidney Function Tests. (25571)

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There is need for a rapid and non-traumatic method for assessing individual kidney function. Use of I^{131} labeled compounds and external radioactivity measurements over the kidneys appear quite adequate for the purpose. After injection, uptake and clearance by each kidney are measured by means of radioactivity, producing a printed record by use of appropriate instrumentation(1). However, the method used previously was complicated because the diethanolamine salt of I^{131} labeled 3,5-diiodo-4-pyridone-N-acetic acid (Diodrast) was not kidney-specific and because of its hepatic uptake, required careful placement of the probes(2). In addition, I^{131} labeled analogs of sodium 3,5-diacetamido-2,4,6-triiodobenzoate (Hypaque), methyl-glucamine 3,5-diacetamido-2,4,6-triiodobenzoate (Renografin), sodium 3-acetamido-2,4,6-triiodobenzoate (Urokon), and sodium 3,5-dipropionylamino-2,4,6-triiodobenzoate (Miokon), all have the disadvantage of being cleared slowly by the kidneys, prolonging test time to 15-25 minutes. Although they are essentially not picked up by the liver, they differentiate less clearly the function of the individual kidneys. Theoretical considerations indicated that sodium o-iodohippurate (Hippuran) had the desired characteristics for comparing separate kidney function, namely rapid and complete removal from the blood by the kidneys only. This compound containing I^{131} was then prepared by the 2 methods described and proved quite satisfactory for the purpose.

Methods. Hippuran containing I^{131} was prepared by 2 methods of exchange. In *Method I,* ca. 20 mc I^{131} was released from NaI^{131} solution (Oak Ridge) by 0.33 ml 0.01 M KI, 0.1 ml M $NaNO_2$ and 0.2 ml 2.5 M HCl and the iodine shaken out with 2 ml CCl_4. The shakeout was repeated with the same quantities of reagents but no I^{131}. The combined CCl_4 solutions of I^{131} were washed by shaking with 2 ml H_2O, then extracted with 0.5 ml H_2O plus 0.6 ml 0.1 M NaOH followed by 0.5 ml H_2O plus 0.2 ml 0.02 M NaOH. The NaI^{131} solutions were added to 363 mg Hippuran dissolved in 2 ml H_2O in 10 ml serum vial and adjusted to pH 6 with 0.1 M HCl and pH paper. The vial was closed with rubber stopper covered on underside with Saran film, sealed with aluminum seal and heated 2 hours in boiling water. The contents were transferred to 20 ml beaker and evaporated to dryness, redissolved in 1 ml boiling H_2O and transferred to 12 ml centrifuge tube. The o-iodohippuric acid was then precipitated with 0.6 ml (1 + 9) HCl, centrifuged 2500 rpm/10 min., the supernate removed, the precipitate dissolved in ca. 4.5 ml boiling H_2O, 1 mg KI in 0.1 ml H_2O added and solution cooled in ice water and centrifuged. The precipitate was transferred using a minimum amount of supernate to a small sintered glass filter, washed with small portions of ice-cold saturated solution of non-radioactive o-iodohippuric acid and dried to constant weight at 100°. The yield was 55% of the weight of Hippuran taken and specific activity 49 μc/mg. The m.p. was 173.5° (corr.), which differed from that reported by Novello(3) but agreed with another source which reported 171-174°(4). In *Method II,* I^{131} was released by same quantities of reagents as in Method I into 2 ml chloroform. The chloroform extracts were water-washed and added to 363 mg Hippuran and 10 μg ICl in 0.1 ml $CHCl_3$, in 5 ml absolute alcohol in 30 ml ground glass neck flask equipped with reflux condenser. The mixture was refluxed for 8-9 hours, the condenser removed and evaporated to dryness. The residue was dissolved in 1 ml boiling H_2O, transferred to 12 ml centrifuge tube, the acid released and purified by recrystallization as in Method I.

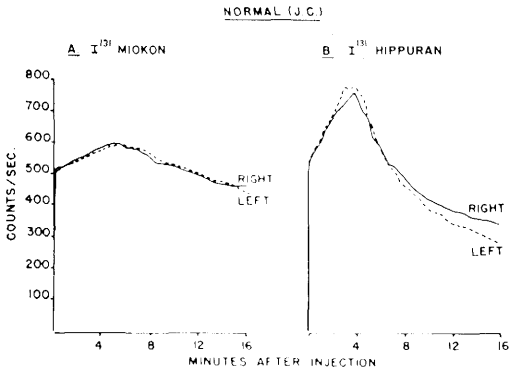

FIG. 1. Comparison of clearance of I^{131} labeled Miokon and I^{131} labeled Hippuran by normal human kidneys.

When dried to constant weight, had same properties and had a specific activity of 65 μc/mg, and same melting point. Solutions containing 25 μc/0.75 ml for parenteral use were made by dissolving requisite quantity of the o-radioiodohippuric acid in stoichiometric amount of 10% Na_2CO_3 solution, diluting with sterile pyrogen-free H_2O, filtering through sintered glass and sterilizing in stoppered and sealed serum vials in boiling water for 30 minutes.

Results. Radioiodinated Hippuran of high purity and adequate specific activity has been prepared by 2 methods. After doses of ca. 25 μc were injected into normal individuals and patients with suspected renal pathology, radioactivity over each kidney area was recorded by 2 matched scintillation probes, ratemeters, and a dual rectilinear recorder. A typical pattern for a normal human, using I^{131} labeled Hippuran, is shown in Fig. 1B, which demonstrates individual kidney function. The more rapid clearance of I^{131} Hippuran compared with I^{131} labeled Miokon is shown in Fig. 1.

The curves are almost identical for right and left kidneys, indicating no uptake by the liver, which would have affected the right kidney reading (Fig. 1B). Additional confirmation was provided by the virtual absence of radioactivity in bile obtained from a common duct T-tube in a patient with normal kidney and liver functions. The lack of liver uptake permitted placement of probes perpendicularly to patient's back, directly over the kidneys, greatly simplifying collimation. Since I^{131} labeled Hippuran is removed by the kidney more rapidly and completely than I^{131} labeled Miokon, Hypaque and Renografin, testing time was reduced to less than 10 minutes. The rapid extraction magnified the absolute function and differences between the 2 kidneys.

Summary. I^{131} labeled sodium o-iodohippurate (I^{131} Hippuran) has been prepared by 2 methods. This compound eliminates major disadvantages of currently used radioactive substances for individual kidney function testing. There is no complication due to liver uptake. It is more rapidly and completely removed, thereby reducing the time for test by 100% or more. Clinical studies with patients having known urinary tract pathology indicate that I^{131} Hippuran demonstrated comparative differences more sensitively than other labeled "renographic" substances(5).

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