TISSUE DISTRIBUTION OF ^{14}C-, ^{125}I-, AND ^{131}I-DIPHENYLHYDANTOIN IN THE TOADFISH, RAT, AND HUMAN WITH INSULINOMAS

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Carbon-14-diphenylhydantoin (DPH) concentrated maximally in pancreatic islet cells of the toadfish 10 min after its intravenous administration. The islet cell-to-acinar-tissue ratio at this time was 6:1. The islet cell-to-liver ratio was 20:1. Iodine-125-paraiodo-DPH at 10 min in the toadfish showed an islet cell-to-acinar-tissue ratio of 1.7:1 and an islet cell-to-liver ratio of 2.3:1. When ^{14}C-DPH was given to four patients 10–17 min before removal of normal pancreatic tissue and of an insulinoma in three of the patients, the concentration of radioactivity in the insulinoma was never greater than in the pancreas and concentrations in insulinoma and pancreas were always less than in liver in the same individual. Forty-five to 90 min after administration of ^{131}I-DPH, the liver and pancreas were delineated in three patients but the insulinoma was not imaged. Five days after administration of ^{131}I-DPH, the concentration of ^{131}I radioactivity in excised tissue was greater in the insulinomas than in the pancreas of two patients and greater than in liver of one patient but not sufficient to produce positive images of the insulinoma in the pancreas.

Kizer, et al (*1*) have demonstrated that therapeutic concentrations of diphenylhydantoin (DPH) directly inhibit the insulin secretory response to glucose without a demonstrable alteration in the islet's ability to utilize glucose.

Although the tissue distribution of DPH has been studied in animals and man (*2–4*), the concentration of DPH in the pancreas has never been evaluated.

Since the toadfish has islet cell tissue segregated into one or more discrete bodies located in the mesentery with only a capsule of acinar tissue easily separable from the islet tissue, we studied the distribution of ^{14}C-DPH in pancreatic islet cells and other tissues of the toadfish (*5*).

Ten minutes after intravenous injection of the tracer dose of ^{14}C-DPH, radioactivity concentration peaked at 8% dose/gm in pancreatic islet cells with a pancreatic islet cell-to-liver ratio of 20:1. This concentration and target-to-nontarget ratio is substantially higher than that previously achieved with selenomethionine (*6*).

The ratios of relative radioactivity concentration in islet cells to acinar tissue, to blood, to liver, and to skeletal muscle were: 6, 7, 20, and 27, respectively.

We now report (A) relative tissue distribution studies in toadfish, rat, and man (with and without insulinoma) 10–17 min after intravenous administration of either ^{14}C-DPH, ^{125}I-DPH, or ^{131}I-DPH; (B) similar studies with ^{131}I-DPH in man, with insulinomas 5 days after its administration; and (C) attempt to detect the insulinomas with radioisotope camera and rectilinear scanning imaging procedures.

METHODS

^{14}C-DPH. The 5,5-diphenylhydantoin (Dilantin®, DPH) was labeled with ^{14}C at the 4 position (see Fig. 1) and was obtained from New England Nuclear, Boston, Mass. The radiochemical purity was greater than 99% as determined by thin-layer chromatography in a solvent system of methanol:glacial acetic acid (98:2).

Trace quantities of DPH were administered: 10 μCi to each toadfish and rat and 100 μCi to each human. Since the DPH had a specific activity of 5.21 mCi/mmole, i.e., 20.65 μCi/mg, the 100-μCi dose contained about 5 mg of DPH. The labeled DPH was formulated by the University of Michigan Nu-

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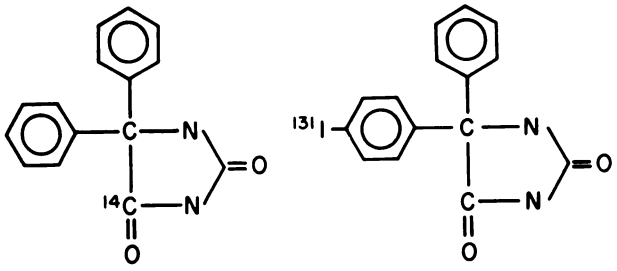

FIG. 1. Chemical configuration of ^{14}C- (left) and ^{131}I-paraiodo-diphenylhydantoin. (right).

clear Pharmacy. The formulation was the same as for commercially available Dilantin® for intravenous injection. To a lot of 250 μCi/12.1 mg, 3 ml of formulation diluent was added giving a specific concentration of 83.3 μCi/ml. The diluent contained 40% propylene glycol, 10% ethanol, q.s. water with pH adjusted to 12 using sodium hydroxide. After dissolution, the product was sterilized by membrane filtration. The administered dose in humans was given in a volume of 1.2 cc. Sterility assurance was ascertained using the BACTEC ionization chamber. Absence of pyrogens was confirmed using the limulus lysate gelatin test.

The solution was administered slowly 10 min before sacrifice of the toadfish or rat and, in the human, after the surgeon had visualized the insulinoma in the operating room, and an estimated 10 min before he excised the insulinoma.

^{125}I- and ^{131}I-DPH. The 5-phenyl-5-(*P*-iodophenyl)-hydantoin was synthesized by Counsell, et al (*7*) (Fig. 1).

The ^{125}I- and ^{131}I-labeled compounds were prepared by the University of Michigan Nuclear Pharmacy with the same formulation as for the ^{14}C-DPH with a specific activity of approximately 100 μCi/mg or about five times the specific activity of the ^{14}C-DPH. A 1-mCi dose of ^{131}I-DPH in the human for in vivo imaging would then contain about 10 mg of DPH.

Toadfish. Mature toadfish of either sex weighing about 1 kg were left in circulating artificial sea water. The tracer dose was injected into a gill arch vein through a 26-gage needle. The fish were sacrificed by a blow on the head at 10 min after a tracer dose of either ^{14}C-DPH (seven fish) or ^{125}I-DPH (four fish) had been given. Immediately the ventral chest was opened and all possible blood was aspirated from the heart chamber. The major islets of the pancreas, usually one, but occasionally two, were removed from the mesentery. The islet cell tissue was isolated from the acinar tissue by dissecting the capsule from approximately 10 mg of the islet cell mass.

Two to 4 mg of acinar tissue (part of capsular connective tissue included) were thus obtained. Two aliquots of tissue samples, weighing about 30 mg each, were obtained with 12 or more other tissues for radioactivity distribution assay.

TABLE 1. RELATIVE TISSUE DISTRIBUTION OF RADIOLABELED DIPHENYLHYDANTOIN IN TOADFISH, RAT, AND HUMAN AT 10 MIN (% DOSE/GM)

	(7) Toadfish	(4)	Rat (4)	Human (1) (Patient No. 5)
	^{14}C-DPH (1.3–5.0 μCi)	^{125}I-DPH (10 μCi)	^{131}I-DPH (18.0 μCi)	^{125}I-DPH (100 μCi)
Islets	7.77 ±1.09	0.7025 ±0.1715	0.83 ±0.05	0.0043* ±0.0003
Acinar	1.29 ±0.33	0.4104 ±0.0609	0.83 ±0.05	0.0060† ±0.0001
Liver	0.39 ±0.02	0.3009 ±0.1182	1.969 ±0.055	0.0074 ±0.0011
Brain	6.22 ±1.30	0.2009 ±0.0556	0.0748 ±0.001	—
Kidney	3.38 ±0.52	0.3618 ±0.1532	0.9684 ±0.0003	—
Heart	2.09 ±0.36	0.3068 ±0.1097	2.0358 ±0.025	—
Spleen	0.22 ±1.04	0.1344 ±0.0354	0.4052 ±0.017	—
Stomach	0.13 ±0.04	0.0186 ±0.0045	—	—
Intestines	0.09 ±0.03	0.1565 ±0.0582	0.4504	—
Muscle	0.29 ±0.05	0.0167 ±0.0038	0.2347 ±0.027	0.0029 ±0.0001
Blood	1.14 ±0.21	0.2928 ±0.0355	0.3126 ±0.0217	0.0057 ±0.0004
Bile	1.12 ±0.06	0.0053 ±0.0014	—	—
Urine	0.02 ±0.01	—	1.3685 ±0.665	—
Lung	—	—	0.4982	—
Thyroid	—	—	3.896 ±0.201	—
Fat	—	—	0.295 ±0.048	0.0008 ±0.0001
Adrenal	—	—	1.0259 ±0.0495	

* Tumor.
† Normal pancreas.

The data obtained from the seven fish given ^{14}C-DPH are from our previous report (*5*) and methods for the ^{14}C radioactivity assay are presented in this paper.

The samples containing radioiodine were dissected free from surrounding fat and connective tissues. Samples were weighed, placed in plastic tubes, and counted in an automatic well counter. Counts were accumulated for a period of time to insure less than 5% statistical counting error and corrections were made for physical radioactive decay and instrument counting efficiency. Standards of the original solution were made and counted identically to the tissues.

TABLE 2. RELATIVE TISSUE DISTRIBUTION OF ^{14}C-DIPHENYLHYDANTOIN IN HUMANS

	(% dose/gm) 100 μCi ^{14}C-DPH at 10–17 min			
	Patient No. 1	Patient No. 2	Patient No. 3	Patient No. 4
Tumor	—	0.0017	0.0068	0.0041
	—	±0.0003	±0.0007	±0.0004
Liver	0.0060	0.0065	0.0079	0.0071
	±0.0000	±0.0013	±0.0001	±0.0004
Pancreas	0.0038	0.0032	0.0067	0.0048
	±0.0003	±0.0005	±0.0003	±0.0008
Muscle	0.0022	0.0020	0.0028	0.0031
	±0.0002	±0.0001	±0.0001	±0.0006
Spleen	0.0023	—	0.0012	0.0020
	±0.0002	—	±0.0004	±0.0002
Fat	0.0008	0.0042	0.0050	0.0017
	±0.0001	±0.0011	±0.0000	±0.0004
Blood (10 min)	—	0.0079	0.0005	0.0043
	—	±0.0022	±0.0000	±0.0008

TABLE 3. RELATIVE TISSUE DISTRIBUTION OF ^{131}I-DIPHENYLHYDANTOIN IN HUMANS

	At 5 days (% dose/gm)	
	Patient No. 2 (708 μCi)	Patient No. 4 (247 μCi)
Tumor	0.0012	0.0009 ±0.0000
Pancreas	0.0008	0.0003 ±0.0001
Liver	0.0019	0.0004 ±0.0000
Muscle	0.0011	0.0001 ±0.0000
Fat	0.0014	0.0002 ±0.0002
Blood	0.0008	0.0021 ±0.0000

Rats. Four Sprague-Dawley rats of either sex weighing approximately 200 gm received radioactive DPH by slow intravenous injection into the femoral vein and were sacrificed under ether anesthesia by decapitation 10 min later. Tissues were sampled and counted as previously described.

Humans. Lugol's iodine (ten drops) was given 2 hr prior to the administration of the dose of ^{131}I-DPH and continued for 7 days. For an attempt at in vivo pancreatic imaging, the ^{131}I-DPH was given by slow intravenous injection, usually 5 days before operation. One patient was given ^{125}I-DPH 10 min before excision of his insulinoma. In the patients operated upon 5 days after a tracer dose of ^{131}I had been given for scanning, ^{14}C-DPH was given 10 min before excision of the insulinoma for tissue concentration studies.

Radioactive DPH was given to five patients as shown in Tables 1, 2, and 3.

Patient No. 1 (JH) was a 21-year-old woman with factitious hyperinsulinism. She was given ^{14}C-DPH only, 16 min before a partial pancreatectomy on November 19, 1973.

Patient No. 2 (ET) was a 48-year-old woman with a 0.5-cm diameter insulinoma weighing 0.5 gm excised from the middle of the head of the pancreas. She was scanned, on January 18, 1974, 90 min after 708 μCi and on January 31, 1974 10 min after 1,500 μCi of ^{131}I-DPH had been given, respectively. On February 5, 1974 she was given 100 μCi of ^{14}C-DPH 10 min before the insulinoma was excised.

Patient No. 3 (JC) was a 52-year-old man who was given 100 μCi of ^{14}C-DPH 10 min before a 4-cm diameter insulinoma weighing 10 gm was removed from the tail of the pancreas on February 4, 1974.

Patient No. 4 (HT) was a 44-year-old man scanned on March 13, 1974 45 min after administration of 247 μCi of ^{131}I-DPH. He was given 100 μCi of ^{14}C-DPH on March 18, 1974, 10 min before a 1.5-cm insulinoma weighing 3.7 gm was removed from the junction of the head and body of the pancreas.

Patient No. 5 (KS) was a 67-year-old man scanned on February 13, 1974, 99 min after intravenous administration of 1,510 μCi of ^{131}I-DPH. Four months later, on June 27, 1974, a 1.0-cm diameter insulinoma weighing 0.35 gm was removed from the junction of the head and body of the pancreas. He received 100 μCi of ^{125}I-DPH 10 min before excision of the tumor. The view of the radioisotope camera was focused anteriorly over the patient's abdomen in the region of the pancreas and continuous pictures were taken and recorded on the disk of an MDS computer with integration times of 5 min each to a total of 45 min.

The details of the use of the camera and the scanner for pancreas scanning have been published previously (*8*). The rectilinear scanning was performed with a dual-headed 5-in. crystal and a collimator with a focal length of 4 in. The pancreas is approximately one half the distance from the anterior wall of the abdomen to the back in the midline and two thirds the distance from front to back to the left of the midline (*9*).

RESULTS

Tissue distribution. Table 1 summarizes the relative tissue distribution of ^{14}C-DPH in seven toadfish 10 min after its administration as published previously (*5*). The peak relative and absolute concentration of radioactivity in pancreatic islet cells from

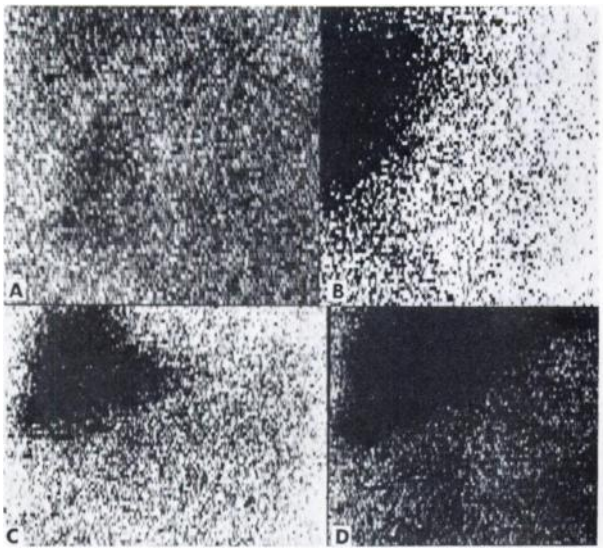

FIG. 2. Pancreas scans: (A) 90 min after administration of 708 μCi of ^{131}I-DPH to patient No. 2, (B) 45 min after administration of 247 μCi of ^{131}I-DPH to patient No. 4, (C) 15 min after administration of 250 μCi of ^{75}Se-selenomethionine to patient No. 5, and (D) 99 min after administration of 1,510 μCi of ^{131}I-DPH to patient No. 5.

^{14}C-DPH was seen at 10 min. The islet-to-acinar tissue ratio at this time was 6:1. The islet cell-to-liver ratio was 20:1.

Table 1 also shows relative tissue distribution of radioactive DPH at 10 min in toadfish, rat, and one patient. The islet cell-to-acinar tissue ratio in toadfish is 1.7:1. Islet cell-to-liver ratio was 2.3:1.

Table 1 also shows the ^{131}I-DPH tissue distribution study in four rats at 10 min. The concentration ratio of radioactivity at 10 min in the pancreas as compared with the liver is approximately 0.5.

The concentration of ^{125}I-DPH in islet cell tumor, in pancreas, and in liver at 10 min after the dose in patient No. 5 was significantly greater than in all other tissues including blood. There was no greater concentration in islet cell tumor than in the rest of pancreas or liver, however. In this patient 48% of the dose was excreted in the urine in 1 day after ^{14}C-DPH and 29% in 3 days after ^{131}I-DPH.

Table 2 presents the tissue radioactivity distribution data on the four patients 10–17 min after ^{14}C-DPH.

The tissue concentration of ^{14}C-DPH 10–17 min after its administration was never greater in insulinoma than in pancreas and always less than in liver in the same individual. The blood concentration was higher than in tumor in patients Nos. 2 and 4 but significantly lower in patient No. 3.

The concentration of ^{131}I-DPH 5 days after its administration is significantly greater in insulinoma than in pancreas (and greater than in liver in patient No. 4) as shown in Table 3. The concentration at this time, however, for these doses of less than 1 mCi of ^{131}I-DPH probably would not be sufficient to produce a positive insulinoma scan but was probably higher 45 min–1½ hr after the doses when the pancreas scans were performed. The half-time blood disappearance for ^{14}C-DPH was 36.6 hr and for ^{131}I-DPH was 42.3 hr.

The calculated whole-body radiation dose in these patients per 100 μCi of ^{14}C-DPH and ^{131}I-DPH was 8 mrads and 38.1 mrads, respectively.

Scans of pancreas. Figure 2A shows the pancreas scan of patient No. 2, 1½ hr after the tracer of 708 μCi of ^{131}I-DPH. The scan was read as equivocal for delineating the pancreas and no insulinoma was detected in the head of the pancreas where the insulinoma was found.

Figure 2B is the scan on patient No. 4, 45 min after 247 μCi of ^{131}I-DPH. This scan was read as equivocal for visualizing the pancreas and no insulinoma was discretely imaged at the junction of the head and body of the pancreas.

Figure 2D is a scan done on patient No. 5, 99 min after 1,510 μCi of ^{131}I-DPH. The scan was read as definite for visualizing the liver and equivocal for delineating the pancreas. No insulinoma was discretely imaged. Figure 2C is a pancreas scan done 15 min after intravenous administration of 250 μCi of ^{75}Se-selenomethionine for comparison. This was also read as equivocal for delineating the pancreas and no insulinoma was imaged.

DISCUSSION

When the radioactivity from ^{14}C-DPH was found to concentrate in toadfish in an 8% dose/gm of islet tissue 10 min after its administration with an islet cell-to-liver ratio of 20:1 (*5*), it was hoped that a radioiodinated analog of DPH could be developed to demonstrate and localize insulinomas in the human pancreas in vivo. Ninety percent of human insulinomas are less than 3 cm and frequently less than 1 cm in diameter (*10*). As a result, at this institution, 60–70% of insulinomas are not detected by pancreatic arteriography, the most effective alternate method (*10*). Not infrequently the pancreas must be removed subtotally and sectioned serially in order to decide whether or not an insulinoma is present and has been resectioned.

Since we have discretely imaged aldosteronomas as small as 2 cm in diameter with ^{131}I-19-iodocholesterol at depths of 5-in. in the background of normal adrenal gland, it would be a reasonable goal to develop a radiopharmaceutical to concentrate in insulinomas diagnostically. The ortho-, meta- and paraiodophenylhydantoin has been synthesized (*7*). The paraiodo analog dehalogenated sufficiently slowly in vivo to be clinically useful.

In the rat the relative tissue distribution of radioactivity from ^{125}I-paraiodophenylhydantoin simulated the ^{14}C-DPH tissue distribution in that the highest concentrations were in the pancreas, brain, heart, and liver. In man the highest concentration of radioactivity, both after tracer doses of ^{14}C-DPH and ^{131}I-DPH simulated the distribution in rats in that the highest measured concentrations were in pancreas and in liver, 10 min and 5 days after their administration.

These preliminary data plus the data showing that the concentration of ^{131}I from ^{131}I-DPH 5 days after the dose may be significantly greater in the insulinoma than in the pancreas and liver in some patients justify further efforts to enhance the percent dose per gram uptake of ^{131}I-DPH by increasing the specific activity and evaluating the pancreatic uptake between 2 hr and 5 days after the dose.

If we could have obtained better pancreas scans with 708 μCi of ^{131}I-DPH than with 250 μCi ^{75}Se-selenomethionine (Fig. 2A and C), there would have been an advantage in radiation dose. The total-body radiation dose from the ^{131}I-DPH was approximately 336 mrads whereas it is approximately 2,000 mrads from 250 μCi of ^{75}Se-selenomethionine.

Two findings are of interest. Although previously we investigated the possibility that other agents acting upon pancreatic islets to inhibit release of insulin would concentrate in pancreatic islets or acinar tissue (*11–14*), radioiodinated DPH is the first agent to show significant selective concentration in the pancreas. DPH, extensively used clinically as an anticonvulsant and antiarrhythmic agent and causing hyperglycemia and occasionally ketosis, was found in our studies to show the highest concentration in brain, heart, liver, and pancreas.

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