^{125}I-insulin: kinetics of interaction with its receptors and rate of degradation in vivo

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SODOYEZ, J. C., F. R. SODOYEZ-GOFFAUX, AND Y. M. MORIS. *^{125}I-insulin: kinetics of interaction with its receptors and rate of degradation in vivo.* Am. J. Physiol. 239 (Endocrinol. Metab. 2): E3–E11, 1980.—Intravenously injected [^{125}I]monoiodoinsulin (^{125}I-Tyr-insulin) was mainly taken up by the kidney and by the liver. To focus on the saturable mechanisms pertaining to plasma insulin clearance, the following experiments were performed on fed, Nembutal-anesthetized, and nephrectomized rats. Following intravenous injection of ^{125}I-Tyr-insulin alone, the apparent distribution space of the tracer was 100 ml/100 g body wt. The concomitant injection of saturating amounts of insulin (4 U) reduced the tracer's distribution space to 20 ml/100 g. Following intravenous injection of ^{125}I-Tyr-insulin alone, liver uptake of radioactivity was rapid and important. After the 3rd min, liver radioactivity decreased with a half-life of 6.5 min. The concomitant injection of 4 U native insulin markedly reduced liver radioactivity and altered its time course. Following sequential injection of ^{125}I-Tyr-insulin at *time 0* and an excess of native insulin at time *t*, liver radioactivity briskly decreased, but not to the level achieved by the concomitant injection of ^{125}I-Tyr-insulin plus insulin, indicating that some radioactivity was irreversibly bound to the liver. Within 2 min, the radioactivity released by exogenous insulin reappeared in the plasma as intact ^{125}I-Tyr-insulin. The amount of ^{125}I-Tyr-insulin reversibly bound to the receptor compartment decreased with a half-life of 4 min. ^{125}I$^-$ was the major radioactive breakdown product of ^{125}I-Tyr-insulin that reentered the plasma. Production of ^{125}I$^-$ was markedly reduced by saturation of the insulin degrading mechanisms or of dehalogenase activity. In the former case, ^{125}I-Tyr-insulin remained undamaged in the plasma. In the latter case, ^{125}I-Tyr was produced instead of ^{125}I$^-$. As estimated by ^{125}I$^-$ production rate, ^{125}I-Tyr-insulin degradation was initiated shortly after monoiodoinsulin injection and completed within ± 10 min. It is concluded that four-fifths of the extrapancreatic insulin were bound to the liver and peripheral tissues. On a functional basis, two cellular subcompartments were defined, one in reversible equilibrium with the plasma and which contains ^{125}I-Tyr-insulin (receptor) and another not in equilibrium with the plasma and probably concerned with ^{125}I-Tyr-insulin degradation.

insulin distribution space; nephrectomized rats; liver extraction; receptor compartment; nonreceptor cellular compartment; monoiodotyrosine dehalogenation; monoiodoinsulin degradation.

IT HAS BEEN KNOWN for a long time that the biological activity of an insulin derivative influenced its metabolic clearance rate. This has been particularly well documented for heavily radioiodinated insulin (3, 13, 18, 22, 23, 26, 32, 35). It was also known that the injection of native insulin increased the plasma concentration of previously injected radioiodinated hormone (24, 31). These findings were recently confirmed and extended in rabbits injected with labeled low- (guinea pig) or high- (pork) affinity insulin or sequentially injected with labeled and native high-affinity insulin (36). Concomitant injection of native and labeled insulin decreased the distribution space (DS) or both the DS and disappearance rate from the plasma (30, 36) of the labeled tracer. Interpretation of these results lead to the conclusion that a sizable receptor compartment was demonstrable in vivo (30, 36). Most of these studies were based only on measurement of plasma iodinated insulin concentration. In this study, we analyzed the fate of intravenously injected purified [^{125}I]monoiodoinsulin (^{125}I-Tyr-insulin) measuring the time course of both liver radioactivity and plasma radioactive components. As will be shown, a large receptor compartment, characterized by a high hormone turnover rate, is in equilibrium with the plasma. At least half of this receptor compartment was localized in the liver. In addition, analysis of liver radioactivity permitted us to define a second compartment in the tissue, not in equilibrium with the plasma and possibly concerned with insulin degradation.

MATERIALS AND METHODS

Animals. Fed male albino rats weighing 250–300 g were anesthetized with Nembutal (50 mg/kg, ip). Intravenous injections were performed via a catheter inserted into a jugular vein. Blood was drawn by heart puncture. When indicated, bilateral nephrectomy was performed immediately before the intravenous injection(s).

Native insulin. Pork insulin (RI neutral, 40 IU/ml) was purchased from the Leo Co.

[^{125}I]monoiodoinsulin (^{125}I-Tyr-insulin). Purified bovine insulin was labeled with Na^{125}I and the monoiodinated derivative purified by DEAE-A-25 Sephadex chromatography (29). The purified labeled hormone was over 95% precipitable by 5% trichloroacetic acid, 95% adsorbed by dextran-coated charcoal, and 80–85% bound by a guinea pig anti-insulin serum after an overnight incubation at 4°C. Its specific activity was 200–350 μCi/μg. After chromatography on G-50 F Sephadex, 87% of the radioactivity was eluted in the volume of native insulin and 2.5% in the exterior volume. No radioactivity was detectable in the elution volumes of ^{125}I$^-$ or of [^{125}I]monoiodotyrosine (^{125}I-Tyr) (Fig. 1*A*). After oxidative sulfitolysis and separation of the S-sulfonated chains by isoelectric

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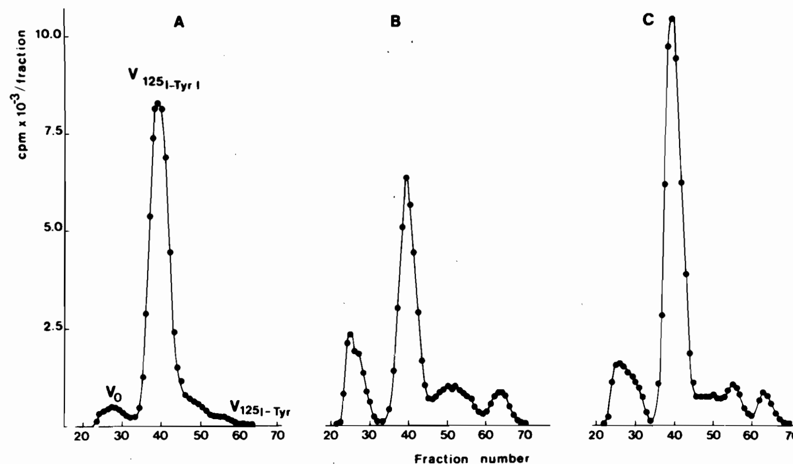

FIG. 1. *A:* [^{125}I]monoiodoinsulin (^{125}I-Tyr-insulin) was mixed with 1 ml rat serum incubated 1 h at 37°C and chromatographed on a 60 x 1.2 cm G-50 F Sephadex column. Two and a half percent of applied radioactivity was eluted in exterior volume (V_o) and bulk of it, in volume of undamaged ^{125}I-Tyr-insulin ($V_{^{125}I\text{-Tyr-insulin}}$). *B:* elution pattern of rat serum drawn 1 min after injection of ^{125}I-Tyr-insulin. *C:* elution pattern of rat serum drawn 3 min after injection of ^{125}I-Tyr-insulin and 2 min after injection of 4 U native insulin. Note that total amount of radioactivity eluted in volume of ^{125}I-Tyr-insulin is greater in *C* than in *B*.

focusing, circa 90% of the radioactivity was associated with the A chain. Following chymotrypsin digestion of the A chain and G-25 Sephadex chromatography of the digestion products, circa 90% of the radioactivity coeluted with the $A_{1\text{-}14}$ peptide. From the above, we may conclude that at least 80% of the iodine was localized on Tyr A_{14}, leaving largely unsubstituted the other tyrosyl residues.

Effect of native insulin on uptake of radioactivity by liver and kidneys. ^{125}I-Tyr-insulin (1.8 μCi, 100–175 μU) alone or mixed with increasing amounts of native insulin (5–5,000 mU) was injected at *time 0.* The rats were killed 3 min later and the specific activity of the liver and kidneys measured.

Time course of liver radioactivity and clearance rate of ^{125}I-Tyr-insulin from plasma. Nephrectomized rats received 2.7 μCi ^{125}I-Tyr-insulin (150–270 μU) at *time 0* and were killed 1–17 min later. The total and trichloroacetic acid (TCA) precipitable radioactivities were measured on a 100-μl aliquot sample of each serum. Two hundred-microliter samples of the sera drawn at the same sampling time were pooled and gel chromatographed on a 60 x 1.2 cm G-50 F Sephadex column developed with 1 M acetic acid that had previously been calibrated with dextran blue T 2000, ^{125}I-Tyr-insulin, $^{125}I^-$, and [^{127}I]monoiodotyrosine (^{127}I-Tyr). The fractions eluted in the volume of undamaged ^{125}I-Tyr-insulin were pooled, lyophilized, and redissolved in 2 ml 0.015 M phosphate buffered saline, pH 7.4. One milliliter of this solution was mixed with 100 μl of a 10% dilution of anti-insulin serum (2 U/ml binding capacity). The other 1-ml aliquot sample was mixed with 100 μl buffer. After an overnight incubation at 4°C, dextran-coated charcoal was added, the tubes were centrifuged, and the radioactivity of the precipitate and supernatant were measured (12).

Some rats received the same amount of radioactive tracer mixed with 4 U native insulin. The experimental procedure was the same as above except that 100 μl of undiluted anti-insulin serum were used to determine the percentage of immunoreactive radioactivity.

The liver was immediately removed and cut into 0.5- to 1-g pieces, and the radioactivity measured.

Total liver radioactivity was expressed as percentage of the total injected.

Reversibility of insulin receptor interaction. Nephrectomized rats received the same amount of ^{125}I-Tyr-insulin alone at *time 0* and 4 U of insulin 2 or 4 min before they were killed. One hundred microliters of undiluted anti-insulin serum were used to determine the immunoreactivity of the material eluted in the volume of a ^{125}I-Tyr-insulin standard.

Evaluation and tissue distribution of dehalogenase activity. Tyrosine (0.1 mg) was labeled with Na^{125}I (2.5 mCi) using hydrogen peroxide and lactoperoxidase (34). The Tyr/^{125}I molar ratio was 485:1, ensuring the formation of monoiodotyrosine. The iodination mixture was applied on a 60 x 1.2 cm G-25 F Sephadex gel eluted with 1 M acetic acid. This procedure, adapted from the work of Lissitzky et al. (17), separated the components of the iodination mixture into unreacted tyrosine (*peak 1*), unreacted iodide (*peak 2*), and ^{125}I-Tyr (*peak 3*).

^{127}I-L-Tyrosine (^{127}I-Tyr, lot no. 1-8250) was purchased from Sigma Chemical Co, St. Louis, MO.

Six rats received an intravenous injection of 7.8 μCi ^{125}I-Tyr alone or mixed with increasing amounts of ^{127}I-Tyr, blood was drawn 3 min later, and 1 ml serum applied on a 1 x 1.2 cm diethyl-(2-hydroxypropyl)aminoethyl (QAE) A-25 Sephadex gel equilibrated with 0.1 M tris glycine buffer, pH 8.5. Nine milliliters of 0.1 M glycine HCl (pH 2.8) eluted all the ^{125}I-Tyr but not the iodide. One M KI in 0.1 N HCl was then applied and the flow stopped for 15 min. Three to 4 milliliters of the latter solution were required to elute the free iodide.

Approximately 0.5 g of various organs were homogenized in 2 ml of 0.015 M phosphate-buffered saline (pH 7.4) to which ^{125}I-Tyr (± 100,000 cpm) was added. After a 1-h incubation at 37°C, the tubes were centrifuged. The pellets contained less than 5% of the total radioactivity and were discarded. The supernatants were analyzed for $^{125}I^-$ and ^{125}I-Tyr concentration using QAE A-25 Sephadex anion exchange chromatography.

Six rats received 6.1 μCi ^{125}I-Tyr-insulin with (experimental group) or without (control group) 25 mg ^{127}I-Tyr. One rat of each group was bled after 3, 6, or 9 min. The

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serum radioactive degradation products of ^{125}I-Tyr-insulin were then analyzed by G-50 F followed by QAE A-25 Sephadex chromatography.

RESULTS

Effect of native insulin on uptake of radioactivity by liver and kidneys. As shown in Fig. 2, the liver specific activity progressively decreased when increasing amounts of native insulin were injected with the tracer.

In the presence of the tracer alone, kidney specific activity was twice that of the liver. By contrast with the liver, kidney specific activity increased when increasing amounts of insulin were injected with the tracer, confirming that the kidney uptake of insulin was a nonsaturable process (4, 10, 11). To more precisely investigate the role of the tissue insulin receptors, including those of the liver, all subsequent experiments were performed on nephrectomized rats.

Time course of liver radioactivity. As shown in Fig. 3, large amounts of ^{125}I-Tyr-insulin were rapidly associated to the liver (43% of the total injected dose after 3 min). After the 3rd min, liver radioactivity rapidly decreased to less than one-fourth its maximum value 17 min after the injection. When an excess of native insulin was injected with the tracer, liver radioactivity was but 10% of the injected dose after 3 min and slowly decreased thereafter.

Clearance rate of monoiodoinsulin from plasma. Following G-50 F Sephadex chromatography, serum radioactivity was fractionated into five components successively eluted in the exterior volume (*peak 1*), in the volume of ^{125}I-Tyr-insulin (*peak 2*), in a volume intermediate between that of ^{125}I-Tyr-insulin and that of ^{125}I$^-$ (*peak 3*), in the total volume (^{125}I$^-$, *peak 4*) and in a volume larger than the total volume (^{125}I-Tyr, *peak 5*).

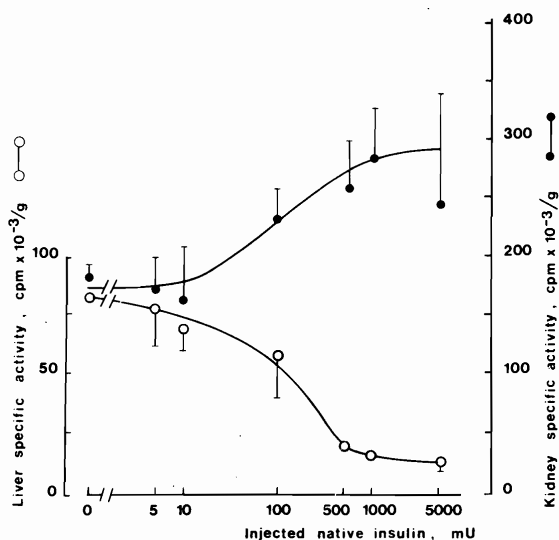

FIG. 2. [^{125}I]monoiodoinsulin (^{125}I-Tyr-insulin) (1.8 μCi) was injected with or without increasing amounts of native insulin. Three minutes after injection, specific activity of liver (○——○) and kidney (●——●) were measured. Each point represents average (± SD) of 3 rats.

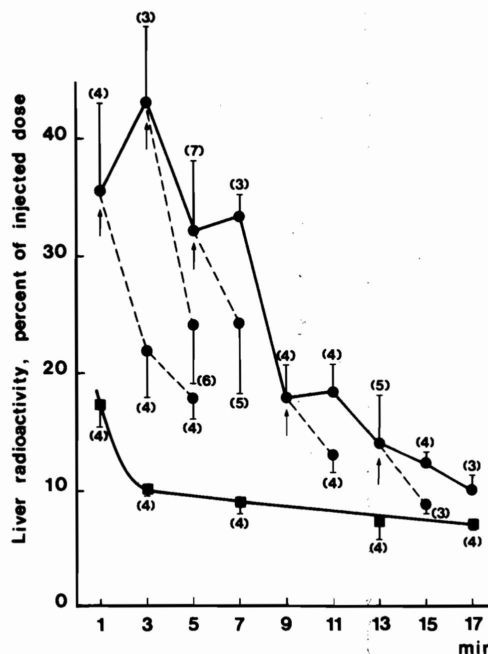

FIG. 3. Time course of liver radioactivity, expressed as percent of total injected radioactivity. Liver radioactivity after intravenous injection of [^{125}I]monoiodoinsulin (^{125}I-Tyr-insulin) alone (●——●); organ radioactivity after intravenous injection of ^{125}I-Tyr-insulin + 4 U native insulin (■——■). Some rats received ^{125}I-Tyr-insulin alone at *time 0*, followed by 4 U native insulin at time indicated by an arrow. Native insulin-induced change in liver radioactivity (----). Number of experiments between parentheses. Vertical bars represent 1 SD.

Peak 1 and *2* radioactivity was totally precipitable by 5% TCA and that of *peaks 3, 4,* and *5* was not. *Peak 2* radioactivity was 75 to 85% bound to an excess of anti-insulin serum; that of *peaks 1* and *3* was not immunoreactive. Because the radioactivity of *peaks 1* and *2* varied independently of each other (Table 1), the TCA precipitation method was judged inappropriate to determine plasma ^{125}I-Tyr-insulin concentration and disregarded.

Figure 4 depicts the evolution of plasma ^{125}I-Tyr-insulin concentration as estimated by gel chromatography. Plasma ^{125}I-Tyr-insulin concentration rapidly decreased during the first 3 min, more slowly and in a semilog fashion after the 5th min.

The concomitant injection of 4 U insulin (Fig. 4) decreased the clearance rate of ^{125}I-Tyr-insulin from the plasma and reduced its apparent distribution space.

Reversibility of insulin receptor interaction. Saturating amounts of insulin (4 U) were injected 1 min after the tracer and the animals were killed 2 or 4 min later. As shown in Figs. 1, *B* and *C* and 5*A*, instead of decreasing from 43,000 to 14,000 cpm/ml as in control animals, plasma ^{125}I-Tyr-insulin concentration rose to 72,000 cpm/ml at 3 min and remained at the same level during the next 2 min. Figure 5*A* shows that less and less ^{125}I-Tyr-insulin was released into the plasma when the time elapsed between ^{125}I-Tyr-insulin and native insulin injection grew longer. The amount of released ^{125}I-Tyr-insulin

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TABLE 1. *Serum radioactive components after intravenous injection of ^{125}I-Tyr-insulin alone or mixed with or followed by 4 U native insulin*

Insulin (4U), Time of Injection, min	Sampling Time, min	Serum Radioactivity, cpm/ml	TCA Insoluble Radioactivity, cpm/ml	Radioactive Components Isolated by Gel Chromatography				
				*Peak 1**	*Peak 2*†	*Peak 3**	*Peak 4*‡	*Peak 5*§
–	1	79,022	66,141	15,962	42,988	13,671		6,405
–	3	33,660	25,749	8,953	14,137	3,097	4,005	3,467
–	5	36,979	20,375	9,060	7,913	3,402	12,351	4,252
–	7	46,408	25,510	8,399	7,750	6,589	18,145	5,569
–	9	45,655	17,988	8,309	6,894	4,657	21,412	4,337
–	11	49,025	20,051	8,481	6,275	5,687	23,875	4,657
–	13	49,056	19,671	9,026	6,083	3,924	25,951	4,123
–	15	42,900	15,057	9,095	3,987	4,633	21,278	3,904
–	17	44,626	17,493	9,728	4,462	3,258	23,161	4,016
+ (0)	1	264,585	245,799	26,458	214,049	20,108		3,969
+ (0)	3	95,450	81,037	14,767	65,384	12,302		2,996
+ (0)	7	72,925	65,632	10,501	53,381	3,500	2,844	2,698
+ (0)	13	60,533	54,661	10,291	42,555	1,816	3,995	1,876
+ (0)	17	73,875	62,941	12,928	48,462	2,807	6,205	3,472
+ (1)	3	106,637	93,627	16,422	71,980	5,972	7,251	5,012
+ (1)	5	103,895	92,674	15,272	71,064	5,299	6,026	6,136
+ (3)	5	79,573	60,714	12,338	45,993	6,525	9,549	5,172
+ (5)	7	66,110	42,839	10,908	26,444	5,024	18,841	4,892
+ (7)	9	61,981	37,808	9,359	21,135	6,508	20,639	4,273
+ (9)	11	54,640	27,483	8,469	15,845	4,863	20,708	4,699
+ (13)	15	52,993	24,164	9,327	12,347	3,974	24,218	3,126

^{125}I-Tyr-insulin, [^{125}I]monoiodoinsulin; TCA, trichloroacetic acid. * Partly characterized. † Immunoreactive ^{125}I-Tyr-insulin. ‡ ^{125}I$^-$. § ^{125}I-Tyr.

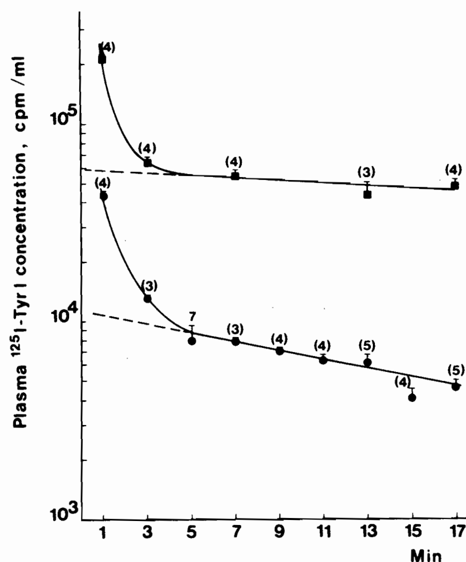

FIG. 4. Plasma concentration of [^{125}I]monoiodoinsulin (^{125}I-Tyr-insulin) after intravenous injection of ^{125}I-Tyr-insulin alone (●——●) or mixed with 4 U native insulin (■——■). Linear portion of curves were extrapolated to ordinate (----) and ordinate intercepts were used to calculate tracer's distribution space. Number of rats between parentheses. Each point represents average (± SD) of *n* determinations.

(plasma ^{125}I-Tyr-insulin concentration of rats that received insulin at time *t* and were killed at time *t* + 2 min − plasma ^{125}I-Tyr-insulin concentration of control animals killed at time *t*) decreased in a semilog fashion as a function of time, with a period of 4 min (Fig. 5*B*).

Native insulin decreased liver radioactivity, even more so when it was injected shortly after the tracer (Fig. 3). It is, however, noteworthy that even when only 1 min elapsed between the two injections, liver radioactivity did not fall to the level achieved by the concomitant injection of excess insulin plus tracer.

Evaluation and tissue distribution of dehalogenase activity. As shown in Fig. 6, 3 min after intravenous injection of ^{125}I-Tyr, free ^{125}I$^-$ already represented 75% of total plasma radioactivity. Liver and kidney radioactivity were low, respectively, 4 and 0.2% of the total injected dose. Increasing amounts of ^{127}I-Tyr decreased the rate of ^{125}I-Tyr dehalogenation. Twenty-five milligrams ^{127}I-Tyr were necessary to saturate the enzyme activity. As shown in Table 2, dehalogenase activity was essentially recovered in the liver and kidney, and to a smaller extent, in muscle and heart. None was detectable in the adipose tissue.

Radioactive end products of ^{125}I-Tyr-insulin degradation. Table 1 shows that the radioactive end product of ^{125}I-Tyr-insulin metabolism was ^{125}I$^-$, the plasma concentration of which increased with time. The injection of excess insulin plus tracer markedly slowed down the rate of ^{125}I$^-$ appearance in the plasma. Saturation of endogenous dehalogenase activity by a previous injection of 25 mg ^{127}I-Tyr almost completely abolished the production of ^{125}I$^-$. Instead of iodine, ^{125}I-Tyr became the predominant circulating radioactive breakdown product of ^{125}I-Tyr-insulin (Fig. 7).

DISCUSSION

Following a bolus injection, ^{125}I-Tyr-insulin was rapidly cleared from the plasma and mainly taken up by two organs, the liver and kidneys. In agreement with others'

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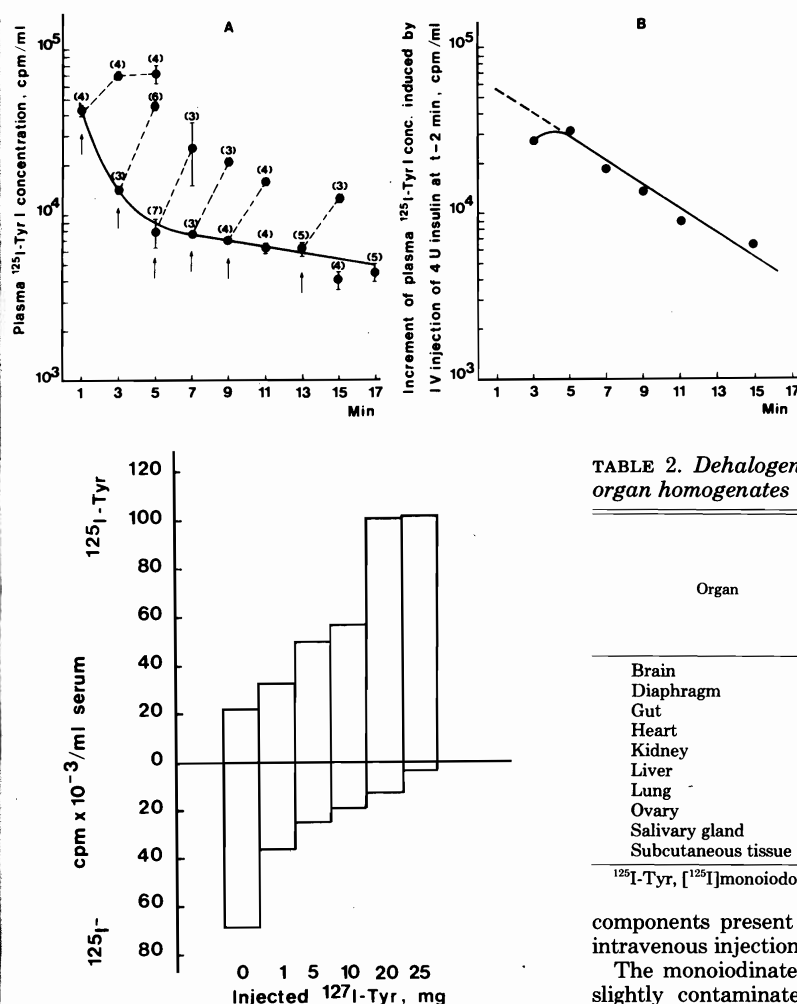

FIG. 5. *A:* plasma concentration of [^{125}I]monoiodoinsulin [^{125}I-Tyr-insulin] after intravenous injection of ^{125}I-Tyr-insulin alone (●——●). In addition, some rats received 4 U native insulin at time indicated by an arrow. Dashed lines represent native insulin-induced change in plasma ^{125}I-Tyr-insulin concentration. Each point represents average (± SD) of n determinations. Number of rats between parentheses. *B:* difference of plasma ^{125}I-Tyr-insulin concentration between rats that received only ^{125}I-Tyr-insulin and were bled at time $t - 2$ min and that of rats that, in addition, received 4 U insulin at time $t - 2$ min and were bled at time t was plotted on a semilog scale as a function of time. Native insulin-induced increments of plasma tracer concentration reflect amount of tracer reversibly bound to tissue receptor compartment.

FIG. 6. Six rats received 7.8 μCi [^{125}I]monoiodotyrosine (^{125}I-Tyr) with or without increasing amounts of [^{127}I]monoiodotyrosine (^{127}I-Tyr) and were bled 3 min later. Serum radioactive components (^{125}I$^-$ and ^{125}I-Tyr) were separated by QAE A-25 Sephadex anion exchange chromatography.

work (4, 10, 11), the kidney uptake of ^{125}I-Tyr-insulin was nonsaturable, and, after 3 min, accounted for 15% of the injected tracer. The liver uptake of labeled hormone decreased when increasing amounts of native insulin were coinjected. In the presence of an excess of native insulin, liver radioactivity was reduced to less than one-fourth its value in the presence of ^{125}I-Tyr-insulin alone. This observation is in keeping with the largely (16, 19, 20, 21, 25) although not unanimously (15) proposed hypothesis that the process whereby the liver removes insulin from the plasma is saturable. To focus on extrarenal mechanisms of insulin uptake and degradation, all subsequent experiments were performed on nephrectomized rats. In this discussion, we shall attempt to analyze the chemical nature and time course of the radioactive

TABLE 2. *Dehalogenase activity in various organ homogenates*

Organ	Wt of Homogenized Tissue, g	Radioactive Components after 1-h Incubation at 37°C of Organ Homogenate + ^{125}I-Tyr	
		^{125}I$^-$ *	^{125}I-Tyr*
Brain	0.47	0.8	99.2
Diaphragm	0.58	8.6	91.4
Gut	0.53	0.1	99.9
Heart	0.49	4.4	95.6
Kidney	0.45	89.5	10.5
Liver	0.30	90.1	9.9
Lung	0.45	0.7	99.3
Ovary	0.16	0.3	99.7
Salivary gland	0.52	0.3	99.7
Subcutaneous tissue	0.53	0.3	99.7

^{125}I-Tyr, [^{125}I]monoiodotyrosine. * Percent of total radioactivity.

components present in the plasma and liver after the intravenous injection of a ^{125}I-Tyr-insulin preparation.

The monoiodinated hormone used in this study was slightly contaminated with high- and low-molecular-weight components (Fig. 1*A*, *peak 1*, eluted in V_o and shoulder of *peak 2*, eluted circa fraction 50). The latter was tentatively identified as A chain because it had the same elution volume after G-50 F Sephadex chromatography, the same isoelectric point (4.4 in the presence of 6 M urea), and the same susceptibility to S-sulfonation (isoelectric point shift to 2.8 in the presence of 6 M urea). Oxidative sulfitolysis of the macromolecular impurity yielded a radioactive product that had the same chromatographic elution profile and isoelectric point as the S-sulfonated derivative of the low-molecular-weight component. We therefore assumed that the macromolecular-weight component was made of A chain linked by disulphide bond(s) to A and/or B chains (aggregates) or to the bovine serum albumin present in the buffer.

Additional purification of the ^{125}I-Tyr-insulin preparation prior to the injection markedly reduced the plasma concentration of these compounds (data not shown). As shown in Table 1, their plasma concentration was high shortly after the injection, decreased over the first 5 min, and thereafter stabilized at an almost constant and equal

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