Physiological Processes and Dynamics in the Disposition of Small and Large Doses of Biologically Active and Inactive ¹³¹I-Insulins in the Rat*

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SUMMARY

The physiological disposition of single intravenous injections of biologically active ¹³¹I-labeled insulin of high specific activity containing an average of 0.8 atom of total iodine per molecule of insulin (mol wt 6000) and of biologically inactive ¹³¹I-insulin containing an average of 6 atoms of iodine per molecule were compared in the rat over a 3-hour interval at two dose levels: tracer or physiological levels (50 to 100 microunits/100 g of rat body weight), and large or pharmacological levels (10⁶ microunits/100 g of rat body weight). Trichloracetic acid (TCA)-precipitable radioactivity was considered to reflect intact insulin specimen, whereas TCA-soluble radioactivity was considered to reflect products of degradation.

Analysis of the data was directed as much as possible at determining the role of fundamental processes such as diffusion, mechanisms of chemical reaction, and transport of species in the distribution of ¹³¹I-insulin and its reaction products in the body of the rat.

The data obtained in this study have been interpreted as indicating that ¹³¹I-insulin species are exchanged between the plasma and various body compartments by unsteady state molecular diffusion processes in which the body compartments behave as semi-infinite homogeneous media.

Superimposed on the diffusional processes are chemical reaction processes occurring in the liver and kidney, with both organs behaving as semibatch reactor systems with a critical volumetric capacity, which must be achieved before significant reaction occurs, of about 3.7 per cent of the injected ¹³¹I dose per g of organ.

The degradation of ¹³¹I species in the liver and kidney appears to occur by a reaction which is second order with respect to the reactant species. Both TCA-soluble and TCA-precipitable species of biologically active ¹³¹I-insulin are degraded in the liver and kidney. Apparent reaction rate constants in the liver are 0.065 and 0.080 (%)-¹ (min)-¹ for TCA-precipitable and TCA-soluble species, respectively; for the kidney the rate constants are 0.030 and 0.014 (%)-¹ (min)-¹ for TCA-precipitable and TCA-soluble species, respectively. (The symbol, (%), denotes percentage of injected dose of ¹³¹I.)

In contrast, biologically inactive ¹³¹I-insulin species appear not to be degraded by the liver and kidney. Present data suggest that the heavily iodinated, biologically inactive ¹³¹I-insulin diffuses into and out of the kidney and liver with little, if any, chemical reaction.

The major muscle mass is indicated to behave as a capacitor which accepts ¹³¹I-insulin species from the plasma when plasma concentrations are high and rejects material to the plasma, for transport to other organs and tissues, when plasma concentrations are low. The existence of degradation reactions in the muscle mass is neither confirmed nor denied by present data.

Concentrations of biologically inactive ¹³¹I species in the plasma, liver, and kidney were found to be independent of dosage level. In contrast, clearance of biologically active, TCA-precipitable ¹³¹I from the plasma was found to be dosage level dependent, this dependence apparently arising from interaction of diffusion and reaction processes. Liver and kidney concentrations of biologically active species were, however, independent of dosage level.

The simultaneous transport and reaction processes are indicated to be quite complex, so that quantitative description of them requires extensive mathematical modeling and data not yet available. A preliminary model based on the results of this study is suggested.

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The disposition of insulin in vivo has been rather extensively investigated both in man (1, 2) and in animals (1, 3-12) with the aid of ¹³¹I-labeled insulin as a tracer. However, the physiological significance of the results may be questioned on the grounds that, in general, the ¹³¹I-insulins used, many of which were prepared commercially (5-12), were of questionable...
integrity, either functionally or structurally (2, 8, 9), or contained unspecified or questionable amounts of total iodine (3, 4, 6-12) attached to the hormone. Furthermore, the low specific activity of the preparations necessitated the injection of large or pharmacological amounts rather than truly tracer amounts of insulin.

The importance of controlling the total amount of iodine attached to insulin was brought out by recent studies in this laboratory (13) which showed that the biological activity of iodoinsulin was markedly influenced by the number of iodine atoms that were attached to the hormone. Full biological activity was preserved only if the iodine content did not exceed, on the average, 1 atom per molecule of insulin (mol wt 6000). With increasing iodination the activity fell off sharply and progressively.

In the light of the above considerations, it was of interest to investigate again the disposition of insulin in vivo with the aid of a structurally and functionally intact 131I-insulin of high specific activity which would permit the injection of small or tracer doses as well as large doses of the labeled hormone. This has been made possible by the recent development in this laboratory of a technique for high level radioiodination of insulin with iodine monochloride (14) that produces virtually no damage to the hormone by irradiation and that permits precise control of the total iodine as well as of the radioactive iodine attached to the hormone.

In the studies to be presented the disposition of intravenously injected single doses of biologically active 131I-insulin of high specific activity was compared with the disposition of a similar 131I-insulin preparation that had been rendered biologically inactive by excessive iodination. Comparisons have been made of the physiological processes and dynamics involved in the plasma clearance, tissue distribution, and degradation of small or tracer doses, as well as of large doses, of the two insulin preparations at various time intervals over a 3-hour period in the rat. As in the studies referred to above, trichloroacetic acid-precipitable radioactivity has been assumed to represent undegraded 131I-insulin, while TCA-soluble radioactivity has been assumed to be derived from the degraded hormone (15). However, because of discrepancies which have been reported in the plasma clearance of 131I-insulin as measured by TCA-precipitable radioactivity in plasma and as measured by isolation of the labeled insulin by paper chromatoelectrophoresis of plasma (8, 9), the plasma clearance of 131I-insulin has been compared by both techniques.

In an attempt to remove residual blood and also any radioactivity in the extracellular compartments of various tissues, each animal killed at an interval of time greater than 1 min after intravenous injection of the 131I-insulin was subjected to a "two way" whole body perfusion (see "Experimental Procedure") with 0.85% sodium chloride solution before the tissues were removed. Because of technical reasons the animals killed at 1 min were not perfused, but appropriate corrections were made for residual blood in the liver and kidneys. The possible role of the plasma itself in the degradation of insulin was investigated by means of a chromatoelectrophoretic study of 131I-insulin which had been incubated in rat blood at 37° for various intervals of time, ranging from 1 min to 3 hours.

The abbreviation used is: TCA, trichloroacetic acid.

EXPERIMENTAL PROCEDURE

131I-Insulin Preparations

For experiments requiring the injection of small or tracer doses of insulin, radioiodinated insulins of high specific activity were prepared according to the method previously described (14). Two types of radioiodinated insulins were prepared. Biologically active 131I-insulins with specific activities of 88 to 112 mC per mg and with average incorporations of approximately 0.8 atom of total iodine per molecule of insulin (mol wt 6000) were prepared by iodinating 150 μg of insulin with 100-mC lots of 131I and with 1 mole of ICl per mole of insulin. Biologically inactive 131I-insulins with specific activities of 200 to 300 mC per mg and with average incorporations of 6 atoms of iodine per molecule of insulin were prepared similarly, but with the use of 8 moles instead of 1 mole of ICl per mole of insulin. For experiments requiring the injection of large doses of insulin the same two types of radioiodinated insulins, but of low specific activity, were prepared. Biologically active 131I-insulin containing an average of 0.8 atom of iodine per molecule of insulin was prepared by iodinating 2.0 mg of insulin with 1 mC of 131I and 1 molar equivalent of ICl. Similarly, biologically inactive 131I-insulins containing an average of 6.0 atoms of iodine per molecule of insulin were prepared by iodinating with 8 moles of ICl instead of 1 mole.

Each preparation of radioiodinated insulin was protected by the addition of human serum albumin immediately after iodination and was stored in lots of 2.0 ml at -18° until ready for use. The preparations were examined for damage by β-radiation by means of paper chromatoelectrophoresis (14). According to this technique damage was minimal in some preparations (less than 5%) and totally absent in others. The radioiodinated insulin in question was thawed shortly before each experiment and was diluted to appropriate concentrations with a cold 0.85% NaCl solution containing 0.6% glacial acetic acid and 1% human serum albumin with a final pH of 3.0. The 131I-insulin preparations of high specific activity were diluted to a final concentration of 200 microunits of insulin per ml of solution, and the preparations with low specific activity were diluted to a final concentration of 200,000 microunits per ml of solution. All dilutions of 131I-insulin were kept in an ice bath up to the time of injection. Three samples (0.1 ml) of the dilution of 131I-insulin to be injected served as counting standards throughout each experiment.

Rats

Female rats of the Wistar strain weighing between 110 and 120 g were purchased from Charles River Breeding Laboratories, Inc., Boston, and were acclimated to their surroundings for several days prior to the experiment. At the start of the experiment, each animal weighed between 150 and 180 g.

Studies in Vivo

Experimental Design—For each series of experiments rats which had been fasted for 18 hours were weighed, placed at random in separate cages, and anesthetized with pentobarbital (3.0 mg/100 g of body weight). During the 18 hours prior to the experiment, the animals were given a 6.25 × 10⁻³ M solution of potassium iodide in tap water instead of tap water to drink, in order to block the uptake of iodide by the thyroid.¹ A sample

¹ This procedure was considered to be effective in blocking the uptake of iodide by thyroid since it was observed that the uptake of radioiodine by the thyroid of the rat following the intravenous injection of 6.0 × 10⁻² μg of Na¹³¹I did not exceed 0.08% of the injected dose.
of blood for determination of blood glucose (control sample) was obtained, 10 min after the injection of pentobarbital, by cutting the tip of the tail. The $^{131}	ext{I}$-insulin was then injected rapidly into the left saphenous vein in a volume of 0.5 ml/100 g of rat body weight. One animal was killed at the end of each of the following time intervals after the intravenous injections: 1, 3, 8, 15, 30, 60, and 180 min. Seven separate series of experiments were performed on different days with small or tracer doses of biologically active $^{131}	ext{I}$-insulin, that is, 50 to 100 micromunits/100 g of rat body weight,$^8$ and five separate series of experiments were carried out with large, hypoglycemic doses, that is, 100,000 micromunits/100 g of rat body weight. Five series of experiments were performed with 50 to 100 micromunits of biologically inactive $^{131}	ext{I}$-insulin per 100 g of rat body weight, and two series were carried out with 100,000 micromunits. With the exception of the animals killed at 1 min, the procedure for which will be described separately, the abdominal cavity of each animal was opened 2 min before the termination of the experiment and, 1 min later, 0.25 ml of heparin (1,000 U.S.P. units per ml) was injected into the vena cava. Any animal which appeared moribund or showed any evidence of circulatory or respiratory distress was discarded. At precisely the designated time of termination of the experiment, the vena cava was incised, a sample of blood was collected, and perfusion was started immediately by cannulating the vein with an 18-gauge hypodermic needle inserted cephalad. The needle was connected by polyethylene tubing to a reservoir containing 0.85% NaCl solution. Urine was collected by inserting a 22-gauge needle, attached to a syringe, into the bladder and removing the contents by suction. As soon as respiration ceased, which was usually a matter of a few minutes, the chest cavity was opened and two cuts were made into the diaphragm. A 20-gauge needle was inserted caudad into the more distal opening, and a second perfusion was started. The two way perfusion of the whole body was carried out for at least 20 min with 500 ml of 0.85% NaCl.

At the completion of the two way perfusion, which was carried out until the tissues appeared to be free of blood, the carcasses were placed on ice and the following tissues were rapidly removed, blotted dry with gauze, and weighed: liver, right gastrocnemius muscle, diaphragm, heart, kidneys, brown fat, and overlying skin. The tissues were then quickly frozen, placed in test tubes, and assayed for radioactivity.

Because of time limitations, the rat killed at 1 min was not perfused. The animal was placed on ice, and a blood sample was removed by cardiac puncture 1 min after the intravenous injection of $^{131}	ext{I}$-insulin. This was followed immediately by rapid dissection and removal of tissues as described above. The removed tissues were thoroughly rinsed with cold 0.85% NaCl solution and patted dry with gauze. The tissues were then weighed, frozen, and assayed for radioactivity. The radioactivities in liver and kidneys were corrected for the radioactivity in the volume of blood estimated to be present in these organs ($^{17}$). However, since the above procedure for handling liver and kidney resulted in some loss of blood, the values are probably over corrected.

Preparations of Blood, Urine, and Tissues for Radioactivity Measurements—The samples of blood taken from the heart or vena cava were placed immediately in test tubes containing an oxalated fluoride mixture and kept on ice. Aliquots were removed for determinations of glucose ($^{18}$) and of radioactivity in whole blood. The remainder of the blood was centrifuged at 4°. An aliquot of the plasma was measured for radioactivity and was then diluted 10-fold with cold 10% TCA and centrifuged at 4°. The supernatant fluid was removed, and the precipitate was washed with 1 ml of cold 10% TCA. Each fraction was measured separately for radioactivity. Serum albumin (0.1 ml of 25% human serum albumin) was added to an aliquot of urine, and the mixture was brought to a final volume of 10 ml with cold 10% TCA and centrifuged at 4° for 20 min at 2000 rpm. The supernatant fluids were decanted and the precipitates were washed with 1.0 ml of cold 10% TCA. The supernatants, washings, and precipitates were each assayed separately for radioactivity.

The frozen samples of liver, kidney, heart, diaphragm, and gastrocnemius muscle were then treated with cold 2% KCl, cut into small pieces in a Petri dish, and then transferred, with the aid of small amounts of 2% KCl, to a homogenizer which had been immersed in an ice bath. Following thorough homogenization, the tissues were transferred into 15-ml centrifuge tubes immersed in crushed ice and were brought to a constant volume with cold 2% KCl. Aliquots (2 ml) of the cold homogenate were treated with 0.2 ml of cold 100% TCA and centrifuged at 4° for 20 min at 2000 rpm. The supernatant fluids were decanted and the precipitates were washed with 1.0 ml of 10.0% TCA. Each of the three fractions was measured for radioactivity.

Method of Assay of Radioactivity—The whole tissues, blood, plasma, and urine were counted for 1 min in a scintillation well detector system, RIDL model, dual channel analyzer with built in amplifier. The supernatants, washes, and precipitates of the homogenized tissues were counted for 10 min. In order to obtain maximal sensitivity, counting was performed with the analyzer in its differential mode. This resulted in an efficiency of 23%, with a background of only 20 to 40 cpm.

Comparison of Plasma Clearance of $^{131}	ext{I}$-Insulin by TCA Precipitation of Radioactivity in Plasma and by Isolation of $^{131}	ext{I}$-Insulin from Plasma by Paper Chromatodeelectrophoresis—Two series of rats were prepared as described above. Each animal received a single intravenous injection of 20,000 micromunits of biologically active $^{131}	ext{I}$-insulin of high specific activity per 100 g of rat body weight. Blood was drawn by cardiac puncture at the end of 1, 3, 8, 15, 30, 60, and 180 min. An aliquot of the plasma was treated with TCA, and the precipitate was assayed for radioactivity as described above. Plasma (10 to 20 ml of the remainder of the plasma) was applied to Whatman No. 3MM paper and chromatodeelectrophoresis was carried out according to a previously reported technique ($^{14}$). The percentage of $^{131}	ext{I}$-insulin per ml of plasma was calculated by the following formula:

\[
\text{Percentage radioactivity} = r_s \times \frac{1000}{(\mu l)_p} \times \frac{100}{(\mu l)_d} \times r_t
\]

where $r_s$ represents the amount of radioactivity remaining at the origin, $(\mu l)_p$ the amount of plasma (in microliters) applied to the paper, and $r_t$ the total amount of radioactivity injected into the animal.
Degradation of $^{131}$I-Insulin in Rat Plasma at 37°—In order to determine to what extent, if any, plasma itself might contribute to the degradation of injected $^{131}$I-insulin, 100 microunits of biologically active $^{131}$I-insulin of high specific activity were incubated with 0.3 ml of normal pooled rat plasma at 37° for 180 min. Aliquots (10 to 20 μl) of the mixture were removed at the end of 1, 3, 8, 15, 30, 60, and 180 min, and were immediately applied to Whatman No. 3MM paper and subjected to paper chromatoelectrophoresis for 15 hours in Veronal buffer, ionic strength 0.1, pH 8.6, according to a previously described technique (14).

RESULTS

Hypoglycemic Effect of Biologically Active and Biologically Inactive $^{131}$I-Insulins—Fig. 1 clearly contrasts the hypoglycemic activity of the biologically active $^{131}$I-insulin, that is to say, the preparations containing an average of 0.8 atom of iodine per molecule of insulin, with the biologically inactive preparations, that is, those preparations containing an average of 6.0 atoms of iodine per molecule of insulin. As anticipated, the heavily iodinated $^{131}$I-insulin preparations manifested little or no hypoglycemic activity, even with doses of 100,000 microunits/100 g of rat body weight. On the other hand, doses of lightly iodinated $^{131}$I-insulin at $10^5$ microunits/100 g of rat body weight produced a profound and prolonged lowering of blood sugar. Furthermore, even what were considered to be very small or tracer doses of biologically active $^{131}$I-insulin (50 to 100 microunits/100 g of rat body weight) appeared to have a slight, transient hypoglycemic effect, as judged by the fact that the mean blood sugar level for the first 8 min following the insulin injections was slightly but significantly lower than the mean levels of blood sugar immediately prior to the insulin injections. The transient early rise in blood sugar that was observed following the injection of the large doses of both biologically active and biologically inactive $^{131}$I-insulin is probably attributable to the presence of small amounts of glucagon in the insulin preparations.

Degradation of Biologically Active $^{131}$I-Insulin by Rat Plasma in Vitro—As measured by its chromatoelectrophoretic mobility on paper in Veronal buffer at pH 8.6 (Fig. 2), biologically active radioiodinated insulin of high specific activity showed no evidence of any appreciable degradation or damage during the first 15 min of incubation in pooled rat plasma at 37°. The radioactivity was largely confined to a narrow zone at the point of application of the $^{131}$I-insulin to the paper, and no appreciable radioactivity was noted in the region of the serum proteins. The results also show that the radioiodinated insulins of high specific activity were free of any appreciable radiation damage. Incubation of the $^{131}$I-insulin for 30, 60, or 180 min in rat plasma resulted in a progressive broadening of the zone of radioactivity at the origin, and in the appearance of increasing radioactivity in the region of the serum proteins. It is reasonable to assume from these studies that, in the experiments in vivo to be described below, plasma did not contribute significantly to the degradation of $^{131}$I-insulin, at least for the first 30 min following the intravenous injection of $^{131}$I-insulin, and that appearance of TCA-soluble $^{131}$I species in the plasma reflected degradation of $^{131}$I-insulin by extravascular tissues.

Plasma Clearance of Biologically Active $^{131}$I-Insulin as Determined by (a) TCA-precipitable $^{131}$I in Plasma, and (b) Separation and Identification of $^{131}$I-Insulin by Paper Chromatoelectrophoresis—Fig. 3 shows that, within the experimental error of the methods, the pattern of plasma clearance of single doses of 20,000 microunits of biologically active $^{131}$I-insulin, as measured by TCA precipitable radioactivity in plasma, was identical with the pattern obtained by the more elaborate procedure of separating and quantitatively determining $^{131}$I-insulin by paper chromatoelectrophoresis. These results indicate that, under the conditions of this study, TCA-precipitable $^{131}$I species in plasma do indeed reflect the behavior of intact $^{131}$I-insulin in plasma, and that the discrepancies which have been reported by others (8, 9) who used the two methods were probably due to the presence of altered components in their $^{131}$I-insulin preparations.

Plasma Clearance, Tissue Distribution, and Degradation of $^{131}$I-Insulins—Data obtained for concentrations of TCA-precipitable and TCA-soluble $^{131}$I in various organs and tissues at selected time intervals after injection of single intravenous doses of biologically active and inactive $^{131}$I-insulin are listed in Tables I through IV. In the ensuing discussion, attention is directed to results for the plasma, liver, kidney, and major muscle mass (represented by the gastrocnemius), since these portions of the body contain or affect nearly all of the accountable fraction of the injected dose.

To illustrate the generally observed change of concentration with time, results for the 50- to 100-microunits, biologically active, TCA-precipitable $^{131}$I-insulin are shown graphically in

**Fig. 1.** Comparison of the hypoglycemic responses to the rapid intravenous injection of single doses of "biologically active" $^{131}$I-insulin, i.e. $^{131}$I-insulin containing on the average 0.8 atom of total iodine per molecule of insulin (mol wt 6000) and "biologically inactive" $^{131}$I-insulin, i.e. $^{131}$I-insulin containing on the average 6.0 atoms of total iodine per molecule. Results are shown for two dosage levels: small or tracer doses (30 to 100 microunits/100 g rat body weight) and large doses (100 microunits/100 g rat body weight). The values are expressed as milligrams of glucose per 100 ml of blood ± 1 S.E.; μU, microunits.
Fig. 2. Effect of preliminary incubation in pooled rat plasma at 37° for various intervals of time on the paper chromatoelectrophoretic behavior of biologically active 125I-insulin. At pH 8.6 undamaged free insulin is adsorbed onto the paper strip at the site of application and remains at the origin when an electrical voltage is applied, whereas the "damaged" or degraded insulin components migrate away from the origin with the serum proteins (14).

Figs. 4 and 5. When these figures are compared it will be noted that the time scale is greatly expanded in Fig. 5 so that the peak in liver concentration which occurs in less than 1 min after injection may be clearly shown. The peak in kidney concentration that is evident in Fig. 4 therefore appears as a plateau in Fig. 5. Note, in Table I and Fig. 5, that data for times less than 1 min after injection were obtained with a dose of 3000 microunits.

The analysis of these data and of those for the other types of doses considered was directed, as much as possible, at determination of the role of fundamental processes such as diffusion, mechanisms of chemical reaction, and transport of species in the distribution of 125I-insulin and its reaction products in the bodies of the rats. Consequently, graphical representations of the data that best illustrate or test these phenomena have been used below. The discussion treats the biologically active and biologically inactive materials separately, because they differ considerably in behavior.

Biologically Active 125I-Insulin—Considering first variation of plasma concentrations, it was anticipated that the decrease of TCA precipitable 125I-insulin concentrations might be controlled by diffusion into other parts of the body, in which case the plasma concentration, $C_{\text{plasma}}$, would be governed by an expression of the general form

$$\frac{dC_{\text{plasma}}}{dt} = D \nabla^2 C_{\text{plasma}}$$  \hspace{1cm} (1)

where $D$ is the effective diffusion coefficient for TCA-precipitable species. Equation 1 cannot at present be applied rigorously to the circulatory system, since the boundary conditions required for integration and a properly representative coordinate system are not yet established. In addition, uptake in the various organs and tissues of the body might occur at different rates and times after injection of the dose. In such circumstances the apparent diffusion coefficient might vary because different parts of the body might cause plasma concentration to decrease at different rates.

Thus, a rigorous solution of Equation 1, to which the data might be compared, cannot at present be obtained. The equation is general for unsteady state diffusion, however, and yields on integration a relationship of the form

$$C_{\text{plasma}} = \frac{1}{\sqrt{4\pi D t}} f(x, t)$$  \hspace{1cm} (2)

where $f(x, t)$ is some function of the coordinate system and time. The explicit form of this function will depend on the geometry of the system and the boundary conditions for integration (21).
Percentage of injected $^{131}$I present in various body compartments at selected time intervals following rapid intravenous injection of single doses of 50 to 100 microunits of biologically active $^{131}$I-insulin per 100 g of rat body weight.

<table>
<thead>
<tr>
<th>Time</th>
<th>Plasma</th>
<th>Liver</th>
<th>Kidney</th>
<th>Muscles</th>
<th>Skin</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Mean mass</td>
<td>TCA-precipitable fraction</td>
<td>TCA-soluble fraction</td>
<td>Mean mass</td>
<td>TCA-precipitable fraction</td>
</tr>
<tr>
<td></td>
<td>ml</td>
<td>%</td>
<td>ml</td>
<td>%</td>
<td>ml</td>
</tr>
<tr>
<td>0.15</td>
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<td>28.3</td>
<td>1.50</td>
<td>12.4</td>
<td>2.0</td>
</tr>
<tr>
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<td>22.0</td>
<td>2.50</td>
<td>13.5</td>
<td>2.3</td>
</tr>
<tr>
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<td>23.9</td>
<td>1.50</td>
<td>13.3</td>
<td>2.5</td>
</tr>
<tr>
<td>0.68</td>
<td>5.5</td>
<td>20.0</td>
<td>2.3</td>
<td>21.3</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Percentage of injected $^{131}$I present in various body compartments at selected time intervals following rapid intravenous injection of single doses of 50 to 100 microunits of biologically active $^{131}$I-insulin per 100 g of rat body weight.

For the commonly encountered situation of diffusion across a fixed boundary into a homogenous, semi-infinite medium, $f(x,t)$ is basically the so-called Gauss error integral. A plot of measured plasma concentrations of TCA-precipitable, biologically active $^{131}$I-insulin with respect to $t$ is given in Fig. 6. The linearity of the relationship for the data obtained with the 50- to 100-microunit dose is, with this representation of the data, strongly suggestive of diffusion-controlled dispersion from the plasma to other body compartments. A plot of Equation 2 in the coordinates of Fig. 3, with $f(x,t)$ given as the Gauss error integral, is linear in the low concentration range.

Further indication of diffusion-controlled dispersion was obtained through consideration of plasma concentrations for times less than 1 min after injection. The data for times of 1 through 180 min after injection were plotted on log-log paper, and the resulting linear curve was extrapolated back through several decades on the time scale to the point at which 100% plasma concentration (i.e. the injection dose) was predicted to exist (Fig. 7). As seen in Fig. 7, the predicted time is about 0.6 sec, and the data obtained for times less than 1 min after injection (Table I) were found to fall on this extrapolated curve within the limits of experimental error. Thus, diffusion-controlled dispersion of TCA-precipitable $^{131}$I-insulin is indicated, for the 50- to 100-microunit dose, throughout the entire time interval studied.

The validity of this interpretation of these data is discussed in more detail below after comparison with results for biologically inactive $^{131}$I-insulin.

Considering again Fig. 6, diffusion-controlled dispersion is not clearly indicated for the 105-microunit dose of biologically active $^{131}$I-insulin. Rather, for times up to about 30 min, higher plasma concentrations but a more rapid rate of concentration decrease were observed (Fig. 7). As seen in Fig. 7, the predicted time is about 0.6 sec, and the data obtained for times less than 1 min after injection (Table I) were found to fall on this extrapolated curve within the limits of experimental error. Thus, diffusion-controlled dispersion of TCA-precipitable $^{131}$I-insulin is indicated, for the 50- to 100-microunit dose, throughout the entire time interval studied.

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the capacity to degrade intact 131I-insulin, the data were examined
from the viewpoint of attempting to determine details of the
degradation process.

Results for the 50- to 100-microunit dose are shown in Figs. 8
and 9, for TCA-precipitable and TCA-soluble species, respec-
tively, as correlations of 1/C with respect to time. The linearity
of the correlations indicates that the data represent an equation
of the form

$$\frac{dC}{dt} = kC^3$$

(3)

where \( k \) is a constant.

Equation 3 describes a chemical reaction system, with \( k \) the
reaction rate constant, which is second order with respect to the
reactant (i.e. 2 molecules are involved in each reaction), and
correspond with any other readily identified reaction mechanism.

The above arguments are clearly quite speculative, and a more
rigorous explanation is beyond the scope of the present study.

The explanation proposed is, however, consistent with other
observed phenomena.

Consider now the results for biologically active 131I-insulin in
the liver and kidney. Since it is known that these organs have
differences are believed to be due to the influence of the reaction
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Equation 3 describes a chemical reaction system, with \( k \) the
reaction rate constant, which is second order with respect to the
reactant (i.e. 2 molecules are involved in each reaction), and
which occurs in a batch reactor. The data can be shown not to
 correspond with any other readily identified reaction mechanism.

It should be noted that Figs. 8 and 9 show only the data for
times equal to or greater than those at which the peak in con-
centration occurs (Figs. 4 and 5). The implications of the
correlations indicates that the data represent an equation
of the form

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where \( k \) is a constant.

Equation 3 describes a chemical reaction system, with \( k \) the
reaction rate constant, which is second order with respect to the
reactant (i.e. 2 molecules are involved in each reaction), and
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Fig. 4. Variation in concentrations of TCA-precipitable $^{131}I$ in plasma, liver, kidney, and muscle with time after intravenous injection of 50 to 100 microunits of biologically active $^{131}I$-insulin. Concentration is expressed as percentage of the injected dose.

Fig. 5. Variation in concentrations of TCA-precipitable $^{131}I$ in plasma, liver, and kidney for short times after intravenous injection of biologically active $^{131}I$-insulin. The peak in kidney concentration shown in Fig. 4 appears as a plateau.

As may be seen by examination of Tables I and II, it was also found that the liver and kidney data for the 105-microunit dose of biologically active $^{131}I$-insulin are identical, within the limits of experimental error, with those shown in Figs. 8 and 9, with respect to both time dependence and concentration expressed as percentage of injected dose. This may be verified with a log-log plot of concentration with respect to time, such as is shown in Fig. 10. The data for the 106-microunit dose show considerably more scatter, however, and have been omitted from Figs. 8 and 9 to preserve clarity.

Many factors relevant to these data are of interest. Considering first the mechanism of the reactions, with apparent rate constants as given in Figs. 8 and 9, the fact that concentrations of TCA-soluble material exhibit the same type of time dependence as the TCA-precipitable material suggests that the liver and kidney degrade not only intact $^{131}I$-insulin but also the reaction products of this initial degradation. Determination of the details of the reactions is beyond the scope of this work. The data indicate, however, the following. (a) Two molecules of reactant are involved in each reaction; (b) the reactant TCA-soluble material is probably composed of a limited number of molecular species (e.g. just the A chain of $^{131}I$-insulin); and (c) the liver makes a much larger contribution to the degradation of both TCA-precipitable and TCA-soluble species, as a result of both higher rate constants and larger mass.

It was previously mentioned that Figs. 8 and 9 are characteristic of a batch reactor, i.e. a closed system in which reaction occurs for a fixed quantity of reactant available at zero time. However, the liver and kidney cannot be, in the usual sense, batch reactors. Reactant-bearing plasma flowed through the organs throughout the period of investigation. Furthermore, peaks in reactant concentration, such as those shown in Figs. 4 and 5, cannot be obtained in a batch system.

This apparent anomaly indicates that phenomena occurring in the liver and kidney are, in fact, quite complex. Further analysis is required, but the present data suggest processes indicated by the following arguments.
The reaction rate constant. The exponent, \( n \), presumably has a value of 2 for present purposes. Similarly, for the soluble species

\[
\frac{dC_s}{dt} = k_s C_s^n + F[C_r(t)] - F[C_s] - k_C C_s^n
\]

which reflects the assumption that soluble species are formed by degradation of TCA-precipitable species.

The existence of peaks in the TCA-precipitable concentrations suggests that a "critical volumetric capacity" must be achieved before a significant amount of reaction occurs. The data indicate that these critical capacities are the same for the two organs. For the liver, the capacity at peak concentration is, from Fig. 2 and Table I, about 21.3/5.6 = 3.8\% of injected dose per g of liver. Similarly, the critical capacity of the kidney is approximately 5.6/1.57 = 3.6\% of injected dose per g of kidney. A further confirmation of the critical capacity concept is the previously noted observation that concentrations in these organs, when expressed as percentage of the injected dose, are the same for both the 50- to 100-microunit dose and the 10^6-microunit dose, even though the latter contains 1000 to 2000 times as many molecules of reactant per unit volume.

After reaction is initiated, concentration changes correspond to the second order batch reactor as previously noted. This behavior may, however, be the net result of a combination of factors. For the TCA-soluble species in particular, a concentration peak occurring in time after the TCA-precipitable peak should exist if it is accepted that the soluble species are degradation products of the TCA-precipitable species and if reaction products are removed from the organ after reaction. If the reaction products are not removed (which is a basic requirement of the batch reactor) the concentration of soluble species should increase with time as more and more of these species are formed by degradation of TCA-precipitable material. Thus the present soluble species data indicate that the liver and kidney do not, in fact, directly correspond to a batch reactor.

A quantitative representation of these phenomena requires, for the TCA-precipitable species, an expression of the form

\[
\frac{dC_r}{dt} = m(t) - F[C_r(t)]
\]

where \( C_r \) is the concentration in the organ at time \( t \), \( F \) is the flow rate (of plasma, presumably) through the organ, \( C_r(t) \) is the concentration of reactant in the incoming plasma, and \( k_r \) is the reaction rate constant.
Utilization of Equations 4 and 5 requires treatment of the liver and kidney as flow or semibatch reactor systems (22). In addition, detailed quantitative analysis to determine the phenomena that produce observed results will require extensive use of a digital or analogue computer and mathematical modeling techniques described below.

Consider now the role of major muscle mass. None of the common chemical reaction mechanisms is clearly indicated by the present data to be operative in this compartment; concentrations of TCA-precipitable material decrease in proportion to t\* throughout the experimental range, as may be verified with a log-log plot of the data in Table I. This time dependence may be the net result of reaction and flow effects in combination, so that, at a fixed point in time, processes appear to be more nearly at completion than would be true if no reaction occurred. As shown below, inactive species apparently do not undergo reaction in the liver and kidney.

The data for both the inactive and the active species are therefore strongly suggestive of diffusion-controlled dispersion of TCA-precipitable species from the plasma. More important, the data together suggest that, for the active species, the experimental time range represents "long time" relative to the inactive species. This is to be expected: the reaction processes that exist for the active species cause changes to occur rapidly, so that, at a fixed point in time, processes appear to be more nearly at completion than would be true if no reaction occurred.

The above discussion emphasizes the fact that the use of only t\* as the abscissa in Figs. 6 and 11 is arbitrary, relative to the actual physical processes involved. When the system is completely defined and necessary physical constants determined it should be possible to show that the influence of diffusional transport on plasma concentrations is the same for all four cases studied (23).

Results for biologically active material in the liver and kidney are shown in Fig. 12 as a plot of concentration with respect to t\* as Figs. 8 and 9. It is apparent that concentrations of biologically inactive material in the liver and kidney obey the laws of diffusion, as shown in Figs. 13 and 14, where concentration is plotted with respect to t\*.

The correlation for inactive species given in Fig. 11 is, throughout the entire range, strikingly similar to the corresponding representation of Equation 2 with f(x, t) as the Gauss error integral (23). A direct analogy cannot be made because the abscissa function involves, for a known system, physical constants which are for the present case unknown. Thus the present abscissa scale should, for analytical purposes, be regarded as arbitrary.

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Although these results show that the liver and kidney affect biologically active and biologically inactive species in markedly different ways, the difference cannot be clearly defined at present. The data suggest limited or negligible reaction of biologically inactive species; however, TCA-soluble inactive species do appear (but at later times after injection than TCA-soluble species; see, for example, Table I as compared to Table III). The sources of this material must be determined, and an analysis such as that suggested above in connection with biologically active species must be made before the causes for observed differences can be clearly established.

Distribution and Degradation of $^{131}$I-Insulin in Other Organs and Tissues—In addition to the results considered in detail above, $^{131}$I concentrations in the heart, diaphragm, and brown fat were also determined at various times following the injections of small and large doses of biologically active and biologically inactive $^{131}$I-insulin. In all of these, the TCA-precipitable and TCA-soluble fractions never exceeded 1 per cent of the injected dose and showed no clearly defined variation with time. Amounts of $^{131}$I urine were also determined, and were found to increase slowly with time after injection, to a maximum of about 3% of the injected dose, for all conditions investigated. Both TCA-precipitable and TCA-soluble $^{131}$I were present.

**DISCUSSION**

It is, in principle, possible that the data for biologically active $^{131}$I-insulin and, consequently, the validity of the interpretation of their significance might be somewhat compromised by two factors: degradation of $^{131}$I-insulin by the plasma itself and the presence of biologically inactive species in the injected dose. Studies discussed above showed, however, that plasma will not significantly degrade $^{131}$I-insulin for at least 30 min. Consequently, the concentration time relationships which, as has been seen, are quite clearly defined for times less than 30 min after injection, may be assumed not to reflect any degradation by plasma. In addition, since the correlations indicated for times up to 30 min apparently also describe the data for times up to 180 min, it is probable that degradation by plasma had negligible effect throughout the entire period of study.

Since biologically active and biologically inactive species were found to exhibit quite different behavior, an analysis of the effect of any biologically inactive species present in the biologically active doses was made. The analysis assumed that any biologically inactive material present would behave as indicated by the results of this study and that the concentration of inactive species in the initial dose was 18%, the maximum considered likely to be present. For these conditions, it was found that data for the active species might be influenced by inactive species only for times after injection greater than 30 min. Since the actual amount of inactive species is probably much less than 18%, it is considered likely that no significant effect of inactive species was present.

Although firm, quantitative statements concerning transport and reaction phenomena cannot be made at this time, the liver appears to dominate metabolic processes for biologically active $^{131}$I-insulin. This domination results from two factors: (a) the large quantity of species handled, and (b) the rapidity of handling, as indicated by the relatively large reaction rate constants and by the short time interval after the injection in which $^{131}$I species clear the liver. In this respect, then, the liver may be considered the prime organ in maintaining insulin homeostasis. In addition, the mechanisms by which liver and also kidney act to

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4 With the aid of experimentally determined radioactivities in the tyrosine residues of insulin, De Zoteen and Van Strik (24) calculated that at an average incorporation of 0.8 atom of iodine per insulin molecule, 41.8% of the molecules were unlabeled, 39.9% contained 1 atom, 15.1% contained 2 atoms, and 2.9% contained 3 atoms, while 0.3% of the insulin molecules contained 4 atoms of iodine. If it is assumed that all molecules containing more than 1 atom of iodine are biologically inactive, then a maximum of 18.3% of the molecules would be inactive. Since, by our technique of iodination, $^{131}$I-insulin preparations containing 1 atom of iodine or less were fully active within the limits of error (±10%) of the methods of assay (33), it is probable that some of the molecules containing more than 1 atom of iodine possessed biological activity or that our method of iodination resulted in fewer molecules containing more than 1 iodine atom.
maintain metabolic processes in these organs do not seem to be affected by 131I concentrations, at least up to those obtained with the 10^4-microunit doses used in these experiments. This is suggested by the fact that the percentages of injected 131I in the liver and kidney not only are the same for both the 50- to 100-microunit dose and the 10^4-microunit dose of biologically active 131I-insulin, but also exhibit the same time function relationships.

The role of the liver and kidney as metabolizing agents is also indicated to extend beyond possible simple production of A and B chain components of 131I-insulin as end product metabolites. The data suggest that the A and B chains, if produced at all, must be subjected to chemical reaction so that the end products of the metabolic processes are fragments of these chains.

This interpretation is supported by the quantitative similarity in the curves of disappearance of biologically active 131I-insulin in plasma as measured by (a) TCA-precipitable radioactivity and (b) chromatoelectrophoretic identification of 131I-insulin (Fig. 3). This would not have been the case if labeled B chain were being released into the circulation as a major metabolite of 131I-insulin. Since B chain, either as the free chain or in complex with albumin, is TCA precipitable, the values obtained by TCA precipitable 131I and TCA soluble 131I are indicated in the columns on the left and right, respectively. Degradation reactions occurring within compartments and transport between plasma and compartments are indicated by arrows. Question marks indicate transport and reaction processes whose existence is at present uncertain.

Fig. 15. Proposed preliminary model of processes involved in the disposition of 131I-insulin in the rat. Compartment for TCA-precipitable and TCA-soluble 131I are indicated in the columns on the left and right, respectively. Degradation reactions within compartments and transport between plasma and compartments are indicated by arrows. Question marks indicate transport and reaction processes whose existence is at present uncertain.

References

because it is convenient and, as a result, will lead to erroneous and insufficiently penetrating interpretation of the data. Use of the biological half-life should be restricted to phenomena which show clearly, for the entire experimental range, a straight line relationship in semilog coordinates. Nonlinear relationships with this form of data representation should be considered to be indicative of processes other than exponential decay.

General Modeling of System—As suggested previously, the results of this analysis indicate a need for a detailed, inclusive model of the body which predicts observed data and which accounts for all physical processes known or believed to occur. The construct of such a model is a difficult and time consuming but not impossible task. Present data indicate some of the details (e.g., second order chemical reaction) that should be built into a model of 131I-insulin dispersion processes; other details will have to be supplied by parametric study with the model itself or additional data.

A preliminary model based on the results of this study is shown schematically in Fig. 15, in which the various compartments known or presumed to exist are shown in a block diagram. Degradation reactions within compartments and transport of 131I-insulin species between compartments are indicated by arrows. The question marks indicate transport and reaction processes not clearly shown by this study to exist. Careful examination of Fig. 15 shows that for each compartment in which reaction occurs, alternative paths for transport of 131I species exist, depending on whether or not the flow paths designated at present by question marks exist. A major function of modeling studies will be to predict the existence or lack of existence of flow paths not now confirmed.

For each compartment, appropriate relationships such as Equations 4 and 5 may be written to reflect known inputs, outputs, and internal processes. The equations may then be solved simultaneously, with the use of flow rates, diffusion coefficients, reaction rate constants and mechanisms, time zero values, etc., as inputs. The output would then be concentration time relationships, such as those determined in this study. By variation of the input parameters, values necessary to match model output to observed data may be determined.

Investigations of this type would utilize an analogue computer, a digital computer, or both. The immediate objective of computer modeling studies related to the present data would be to establish the physical constraints necessary to reproduce the data obtained. An additional benefit would be that the model itself will indicate further data requirements and also suggest paths of research that are likely to be most fruitful. Furthermore, the model ultimately developed can, of course, be tested for applicability to other hormone-dispersion processes.

Investigations involving modeling studies of the type outlined above are planned for the future.

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