JCI The Journal of Clinical Investigation

Amendment history:
- Errata (December 1968)

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Michael P. Stern, … , Abraham Silvers, Gerald M. Reaven

J Clin Invest. 1968;47(9):1947-1957. https://doi.org/10.1172/JCI105884.

Research Article

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Insulin Delivery Rate into Plasma in Normal and Diabetic Subjects

MICHAEL P. STERN, JOHN W. FARQUHAR, ABRAHAM SILVERS, and GERALD M. REAVEN with the technical assistance of RICHARD H. NAKANISHI

From the Department of Medicine, Stanford University School of Medicine, Palo Alto, California 94304

ABSTRACT Removal of insulin-^{131}I from plasma was studied in normal and diabetic subjects with both single injection and continuous infusion of isotope techniques. Patients were studied either in the fasting state or during steady-state hyperglycemia produced by a continuous intravenous glucose infusion. Steady-state plasma insulin concentration during these studies ranged from 10 to 264 μU/ml. Labeled insulin specific activity time curves consisted of more than one exponential, indicating that a multicompartmental system for insulin metabolism exists. A mathematical technique which is applicable to non–first order processes was used to calculate the rate at which insulin was lost irreversibly from the plasma insulin pool. A direct, linear relationship was found between insulin irreversible loss rate and plasma insulin concentration over the range of concentrations studied. This linearity implies lack of saturability of the insulin removal mechanism. Since the plasma insulin pool was in a steady state during these studies, insulin irreversible loss rate was equal to the rate at which newly secreted insulin was being delivered to the general circulation. Therefore, these results indicate that changes in plasma insulin concentration result from parallel changes in the rate of insulin delivery and not from changes in the opposite direction of the rate of insulin removal. A wide range of insulin delivery rates was found among patients with similar plasma glucose concentrations, suggesting that there exists considerable variability in responsiveness to endogenous insulin among these patients.

INTRODUCTION

Development of a precise immunoassay for the measurement of plasma insulin concentration (1) has greatly facilitated study of carbohydrate and insulin metabolism. Interpretation of plasma insulin measurements has generally rested on the belief that changes in concentration are direct reflections of parallel changes in pancreatic insulin secretion rate. However, there is little experimental evidence in support of this assumption. Because insulin is secreted into the portal vein, and not into the systemic circulation, determination of pancreatic insulin secretion rate in intact subjects presents formidable difficulties. In contrast, plasma insulin removal rate, which in the steady state is equal to the rate of delivery of insulin into the systemic circulation, can be determined by standard isotopic techniques, and many such studies have been published (2–8). Unfortunately these studies suffer from a variety of defects. In some cases (3–7) disappearance of nonspecific protein-precipitable radioactivity has been followed despite the fact that Berson, Yalow, Bauman, Rothschild, and Newerly (2), and subsequently others (9, 10) have shown that this technique does not adequately separate intact immunoprecipitable insulin from its labeled degradation products. Fur-

Dr. Stern is a postdoctoral research fellow, U. S. Public Health Service, currently U. S. Air Force Hospital, Andrews Air Force Base, Washington, D. C.

Dr. Silvers is a special research fellow, U. S. Public Health Service, Stanford University School of Medicine, Palo Alto, Calif. 94304.

Requests for reprints should be sent to Dr. John W. Farquhar, Stanford University School of Medicine, Palo Alto, Calif. 94304.

Received for publication 30 June 1967 and in revised form 17 August 1967.

thermore, insulin specific activity time curves are curvilinear when graphed on semilog paper (2–7) indicating that a multicompartmental system exists for insulin degradation. Nevertheless, in none of these studies has insulin removal rate been calculated with mathematical techniques suitable for analysis of non–first order processes. Finally, no study of plasma insulin removal rate includes simultaneous measurements of steady-state immunoreactive insulin concentration, so that in no case has the relationship between removal rate and concentration been defined. In this paper we present a method for analyzing multicompartmental systems which permits calculation of plasma insulin removal rate (which, to repeat, is synonymous with delivery rate), and using this method, we describe the relationship between removal rate and steady-state concentration in normal and diabetic subjects.

METHODS

Experimental subjects. Patients were selected for these studies in order to insure a population with widely varying degrees of glucose tolerance. General description of these subjects and the results of oral glucose tolerance tests (listed in order of decreasing tolerance) are seen in Table I. None of these patients had ever received insulin.

Experimental protocol. Calories were provided in four daily equicaloric feedings of a liquid formula diet. Three diets were used, containing 17%, 42%, and 85% of calories from carbohydrate, respectively. All three diets contained 15% of calories from protein and the remainder from fat. Patients were initially placed on the diet containing 42% of calories from carbohydrates for 1 wk after which an oral glucose tolerance test was performed using 7 oz of a synthetic carbohydrate beverage.[1] Subsequent dietary periods were randomized, and insulin turnover studies were not performed until at least 1 wk, and in most cases until 3 wk, of a particular dietary regimen had elapsed.

All studies were performed after a 15 hr fast. A polyethylene cannula was placed in the brachial artery on the night before the study. The following morning, insulin ^{131}I,[2] 0.75 μc/kg of body weight, was rapidly injected into a peripheral vein, and arterial blood samples

[1] Glucola, Ames Co., Inc., Elkhart, Ind.

[2] Bovine insulin-^{131}I, approximately 4 mc/mg, Abbott Laboratories, North Chicago, Ill.

TABLE I
Clinical Characteristics

Patient	Age	Sex	Height	Weight	Glucose tolerance test*			
					Fasting	1 hr	2 hr	3 hr
			cm	*kg*				
W. W.	56	M	167.6	69.0	65	122	84	62
W. C.	57	M	172.7	67.9	74	136	72	57
B. K.	39	F	169.1	68.4	79	136	104	72
G. L.	45	F	170.0	68.0	102	112	119	74
G. B.	53	M	171.5	79.3	73	166	116	66
L. K.	47	M	180.3	78.6	71	174	122	119
D. H.	51	F	157.4	66.8	76	173	131	110
E. M.	48	M	180.3	73.2	76	191	106	71
C. L.	53	M	175.3	71.2	98	162	125	116
F. B.	59	M	175.3	86.2	104	178	109	85
E. C.	41	M	165.1	90.2	90	201	136	74
W. K.	41	M	174.0	106.0	87	243	169	103
W. G.	48	M	177.8	96.0	106	250	220	119
R. D.	45	M	172.8	94.8	136	193	250	224
E. A.	61	F	154.9	57.9	123	276	292	246
F. H.	68	M	175.3	77.9	148	236	336	244
C. D.	56	M	172.4	69.6	152	320	270	170
J. B.	54	M	168.9	64.0	150	312	380	368
E. D.	58	F	152.4	68.2	205	318	366	342
P. M.	47	F	162.0	111.0	228	364	368	358
D. M.	52	M	168.8	114.8	320	448	486	448
H. H.	68	M	181.6	60.6	304	568	592	496
E. K.	59	F	159.0	67.4	318	490	616	492

* Plasma glucose concentration (mg/100 ml).

were withdrawn 2, 4, 6, 10, 15, 20, and 30 min later and thereafter at 15-min intervals up to 3 hr. In certain instances (glucose infusion studies) glucose was administered intravenously by means of a constant infusion pump at a rate of 7 mg/kg of body weight per minute for 1 hr before the injection of the isotope in order to establish a steady level of hyperglycemia. The infusion was then continued at the same rate for the remaining 3 hr.

In three patients (G.B., W.K., and E.D.), continuous infusions of insulin-^{131}I were given by means of a constant infusion pump. In each of these studies, the continuous infusion of isotope was preceded by a primer dose. The infusion rates were: G.B., 170,686 cpm/min; W.K., 135,240 cpm/min; and E.D., 71,439 cpm/min. Primer doses were in each instance equal to 10 times the per minute infusion rate. Venous blood samples were obtained at ½ hr intervals for 4 hr. Only those samples obtained after isotopic steady state had been achieved were used in the subsequent calculations.

Technical procedures. Blood was drawn into tubes containing ethylenediaminetetraacetate (EDTA). Plasma, separated promptly in a refrigerated centrifuge, was divided into three aliquots for measurement of glucose concentration, immunoreactive insulin concentration, and total immunoprecipitable radioactivity. These aliquots were immediately frozen in acetone–dry ice and stored until analyzed. Plasma glucose was measured by means of a Technicon AutoAnalyzer (11). Disappearance of immunoprecipitable radioactivity from plasma was determined by the method of Grodsky and Forsham (12, 13) in which plasma is reacted with guinea pig anti-insulin antibody and the resultant antigen-antibody complex precipitated with 25% sodium sulfate. This method was modified by omitting the initial acid alcohol extraction and by carrying out the reaction in the presence of excess anti-insulin antibody. Under these conditions, percent recovery of immunoprecipitable radioactivity was greater than 95% over a wide range of "cold" porcine insulin concentrations (10–1000 μU/ml). The total immunoprecipitable radioactivity injected in each experiment was determined by applying the above method to aliquots of injected material saved from each experiment. After sufficient time had elapsed for radioactive decay of the trace amounts of exogenous insulin-^{131}I, the endogenous insulin concentration of every sample was determined in triplicate by a modification of the method of Hales and Randle (14), with insulin-^{125}I and insulin-binding reagent obtained from the Radiochemical Centre, Amersham, England. Since introduction of the labeled insulin resulted in a transient and slight elevation in plasma insulin concentration which died out by 30 min, the steady-state insulin concentration for each experiment was determined from the mean insulin concentration of specimens obtained between 30 and 180 min. Specific activity was calculated by dividing the concentration of immunoprecipitable radioactivity (cpm/ml) in each specimen by the mean concentration of "cold" immunoreactive insulin (μU/ml) for that experiment, providing a figure of cpm/μU.

Analysis of data

When a radioactive tracer is introduced into a single well-mixed pool at time zero, the decay of specific activity in that pool occurs by a first order process which is described by the equation:

$$SA_t = SA_0 e^{-\lambda t} \quad (1)$$

in which SA_t equals the specific activity of the pool at time, t. SA_0 equals the specific activity of the pool at time zero, and λ equals the fractional turnover rate of the pool. When SA_t is plotted against time on semilog paper, a straight line is obtained.

However, all of the insulin-^{131}I time curves which we obtained were curvilinear on semilog paper. Fig. 1 shows an example of such a curve obtained on patient R.D. In analyzing curved functions of this type, it is common practice to attribute the early portion of the curve to "mixing" of the radioactive tracer throughout its volume of distribution and the late portion of the curve to its metabolic removal. The late portion of the curve is extrapolated to time zero, and the resulting straight line is then analyzed using equation 1. This type of analysis is suitable, however, only when mixing is rapid relative to metabolic removal. When both of these events proceed at similar rates, a smoothly curvilinear function is obtained which shows no definite break to indicate the point at which mixing is complete and metabolic removal has begun. Under these circumstances, a considerable portion of the labeled material will have left the system before the completion of mixing so that the pool size which is then calculated by the standard isotope dilution method will be overestimated. This error will tend to produce an overestimate of metabolic removal rate. In addition Baker, Shipley, Clark, and Incefy (15) have shown that it is also possible for underestimates of the metabolic removal rate to occur when the extrapolation technique is used. Although it is possible that in a particular instance the two types of errors may balance one another, it is not possible to determine in a given situation whether or not this has, in fact, occurred.

An alternative method for analyzing curvilinear specific activity time curves is available. Curvilinearity results because radioactive material, before its irreversible loss from the pool being sampled, enters one or more other pools which exchange with the sampled pool. These secondary pools, unlabeled at the outset of the experiment, gradually become labeled so that recycling of the radioactive material back into the sampled pool occurs. Thus, only a portion of the material leaving the sampled pool per unit time is lost irreversibly from that pool. The amount of radioactivity lost irreversibly from the sampled pool during a short time interval, dt, is given by:

$$\lambda_i q(t) dt \quad (2)$$

in which $q(t)$ equals the amount of radioactivity in the sampled pool at time, t, and λ_i equals the fractional irreversible loss rate from the sampled pool. The sum of all such amounts of radioactivity irreversibly lost eventu-

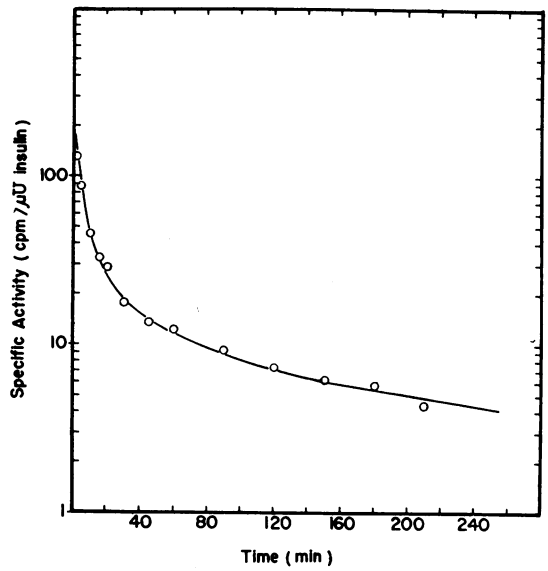

FIGURE 1 Representative specific activity time curve of immunoprecipitable insulin (patient R.D.).

ally equals the total amount of radioactivity introduced into the sampled pool at time zero, so that:

$$\text{injected dose} = \lambda_i \int_0^\infty q(t)dt. \tag{3}$$

If Q is the amount of unlabeled material in the sampled pool, and if steady-state conditions with respect to unlabeled material exist, so that Q is constant, then:

$$\text{injected dose} = \lambda_i Q \int_0^\infty \frac{q(t)dt}{Q}. \tag{4}$$

Since $q(t)/Q$ equals SA_t:

$$\text{injected dose} = \lambda_i Q \int_0^\infty SA_t dt. \tag{5}$$

$\int_0^\infty SA_t dt$ is the area under the specific activity time curve plotted on ordinary coordinates and expressed in the appropriate units. This area may be determined by a variety of techniques; the one which we have employed has been planimetry. Since the injected dose is known, equation 5 permits the calculation of $\lambda_i Q$, which represents the irreversible loss rate of unlabeled material from the sampled pool. Of critical importance is the fact that irreversible loss rate from the sampled pool can be calculated without a detailed knowledge of the size of any of the pools in the system, including the sampled pool, or their manner of interaction with the sampled pool. It should be made clear that in equation 5 we are applying methods to insulin kinetics analogous to those used by other workers in study of other problems. For example, Tait (16) used a similar formula to calculate "metabolic clearance rate" and "production rate," and Zierler (17) had used a similar formula for calculation of cardiac output. Since we have used methods similar to those Tait applied to production or clearance rates of hormones present in plasma (16), our differing choice of terminology bears some discussion. We have rejected the term "production rate" because we believe it fails to distinguish clearly between the total pancreatic secretion rate of insulin and the rate of its delivery into the general circulation. The term "production rate in blood" could be used but seems cumbersome. The term "metabolic clearance rate" seemed inadequate on two counts. First, it fails to exclude from consideration that portion of the total insulin secreted which is cleared by the liver before its entry into the general circulation. Second, the term clearance as used by Tait refers to a volume flux, whereas we measure a mass flux. Therefore, we believe the terms "irreversible loss rate from the sampled pool" or "delivery rate into the sampled pool" describe most accurately and in the most general way the rates under consideration. It should be noted that the first use of the term "irreversible loss rate" may be found in reference 15 as applied to glucose kinetics.

Although equation 5 is preferred to equation 1 on theoretical grounds, under certain circumstances, i.e., when mixing of the radioactive tracer throughout its volume distribution is rapid relative to metabolic removal, both types of analyses will give similar results. However, inspection of Fig. 1 clearly reveals that gradual curvilinearity is present in the case of insulin removal. Therefore, one would predict that equation 1 and equation 5 would give different results. This indeed was found (the differences were statistically significant at the $P < 0.001$ level), and accordingly our results are presented as calculated by equation 5, with the area under the specific activity time curve having been determined by planimetry.

Loss rates were also calculated from equation 5 with the estimate of $\int_0^\infty SA_t dt$ obtained from a particular computer-based method of multicompartmental analysis devised by Berman, Shahn, and Weiss (18). In this method only a minor amount of the potential of this method is used, and the loss rate calculation is in fact made without a need to define the fine structure of the multicompartmental system of insulin kinetics. One proceeds as follows: (*a*) by inspection of the data (Fig. 1 as an example) it is apparent that a two or more exponent system, and therefore at least a two-pool system, exists. When tested in the computer program (18) a three-pool and a two-pool system were equivalent in goodness of fit, and therefore the simpler two-pool system was used. By curve peeling, slopes and intercepts may be calculated and initial estimates for fractional turnover rates obtained for the two-pool system. Details of such a procedure are more completely outlined in a recent paper by Barrett, Berk, Menken, and Berlin (19), which has described a two-pool system for bilirubin kinetics in man (19). These initial estimates and an estimate of plasma insulin pool size (4.5% of body weight times plasma insulin concentration) are entered into the computer program. Final estimates for fractional turnover rates are then obtained by this program by an iterative procedure which finds a best fit for the data through a nonlinear least squares technique (18). The final esti-

mates of fractional turnover rates (λ_{ij}'s) are then converted to a figure identical to $\int_0^\infty SA_t dt$ as follows:

$$\int_0^\infty SA_t dt = SA_0 \left(\frac{\lambda_{02} + \lambda_{12}}{\lambda_{21}\lambda_{02}} \right) \quad (6)$$

in which λ_{ij} is the fraction of insulin in compartment j that is transferred per minute to compartment i. The notation 1 refers to pool 1, 2 to pool 2, and 0 to the site of removal. (No further definition of these pools is needed when this computer program is used for this comparatively minor task.)

We have also calculated irreversible loss rate from the sampled pool from studies in which labeled insulin was infused continuously. These calculations are based on the assumption that, during the isotopic steady state, the irreversible loss rate of radioactivity from the sampled pool is equal to the rate at which radioactivity is being infused. The ratio of irreversible loss rate of labeled insulin to the irreversible loss rate of unlabeled insulin is equal to the ratio of the concentration of the two species of insulin in the sampled pool, i.e., the specific activity of the plasma insulin. Thus:

$$\frac{\text{infusion rate of labeled insulin}}{\text{irreversible loss rate of unlabeled insulin}} = \text{insulin specific activity.} \quad (7)$$

Since the infusion rate of labeled insulin is known, and the plasma specific activity of insulin is measured, equation 7 may be used to calculate the irreversible loss rate of unlabeled insulin.

RESULTS

Introduction of insulin-^{131}I produced a transient disturbance in the plasma insulin steady state. This disturbance is relatively less significant in the glucose infusion studies since in these studies the endogenous plasma insulin pool had been greatly expanded and, therefore, the administered insulin represented a considerably smaller fraction of that pool. In the fasting studies, the transient elevations in plasma insulin concentration were largely gone by 30 min and in many cases even earlier. In order to eliminate the effect of this transient elevation on the calculation of irreversible loss rate, we have used as the mean insulin concentration for each study, the mean of the insulin concentrations obtained between 30 and 180 min after the injection of isotope. Table II compares the mean plasma insulin concentration between 30 and 180 min for the fasting studies with the corresponding plasma insulin concentration obtained before the injection of isotope. A paired t test showed that there was no significant difference between the two concentrations at the $P = 0.05$ level. The average coefficient of variation of the individual concentrations between 30 and 180 min was 28%. Although this variance is admittedly somewhat large, the lack of significant difference between the preinjection level and the postinjection mean indicates that the plasma insulin pool was in a reasonably steady state for most of the duration of each study.

TABLE II

Comparison between Preinjection Plasma Insulin Concentration ($\mu U/ml$) and Mean Plasma Insulin Concentration ($\mu U/ml$) from 30 to 180 Min after Injection of Isotope for the Fasting Studies

Preinjection	30–180 min mean
9	10
12	16
21	22
24	25
27	22
42	29
30	29
18	31
42	31
21	33
18	26
51	34
34	34
54	31
12	27
36	41
12	37

The specific activity by 180 min had in every case declined to less than 10%, and in most cases to less than 5% of the specific activity at 2 min. In order to further minimize the error introduced in the estimation of the area under the curve, we have extrapolated each curve beyond 180 min to the point at which it apparently "touches" the horizontal axis. A more detailed treatment of the magnitude of the error in the measurement of irreversible loss rate by this technique is presented in the Discussion.

Irreversible loss rate of insulin from plasma ranged from 1200 to 10,455 μU/min (0.07–0.63 U/hr) for the fasting studies and from 3320 to 37,818 μU/min (0.20–2.27 U/hr) for the glucose infusion studies. The relationship between plasma insulin irreversible loss rate and insulin concentration is shown in Figs. 2 and 3. A strong linear correlation existed between these two variables ($r = 0.861$; $P < 0.001$ for fasting studies; $r =$

0.852; $P < 0.001$ for glucose infusion studies). The slopes of the two regression lines were not significantly different, since the hypothesis that they were different could not be rejected at the $P < 0.05$ level (t test for significance of difference between slopes). Thus, a single linear relationship exists between these two variables throughout the entire range of insulin concentrations studied. Previous dietary history does not appear to influence this relationship. Since the plasma insulin pool was considered during a steady state, irreversible loss rate may be presumed to equal the rate at which newly secreted insulin was being delivered to the general circulation (delivery rate). Thus, changes in plasma insulin concentration are associated with corresponding changes in insulin delivery rate and not with changes in the opposite direction of insulin removal rate. The apparent linearity of this relationship implies that the insulin removal mechanism does not become saturated over the range of insulin concentrations studied. Also shown in Figs. 2 and 3 are the irreversible loss rates calculated from the three studies in which labeled insulin was infused continuously. It is seen that the results of these

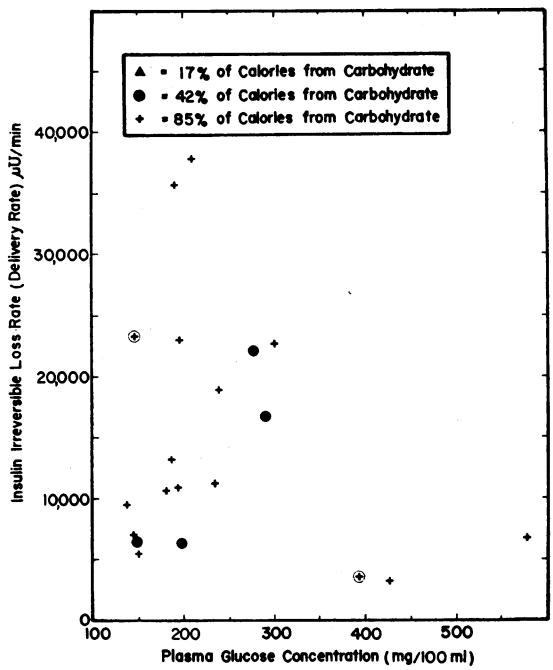

FIGURE 2 Relationship between plasma insulin concentration and insulin delivery rate during fasting. Circled characters refer to studies performed using continuous infusion of isotope technique.

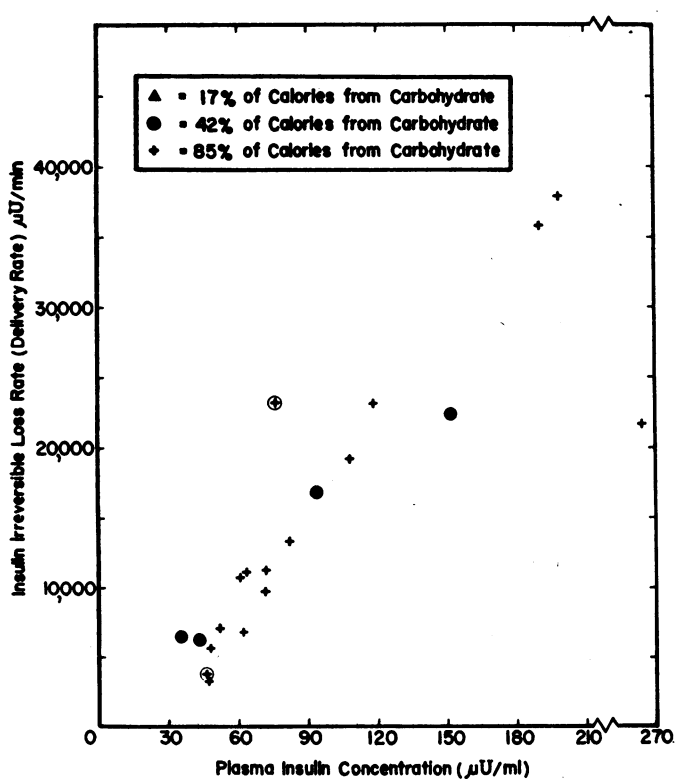

FIGURE 3 Relationship between plasma insulin concentration and insulin delivery rate during continuous glucose infusion. Circled characters refer to studies performed using continuous infusion of isotope technique.

studies fall generally on the same concentration loss rate line, indicating that both types of experiments give similar results. One patient (G.B.) was studied during the fasting state by the continuous infusion technique, and a month later, by the single injection technique. The loss rates calculated from the two studies were 6279 and 5698 μU/min, respectively. Also, as expected, the mean plasma insulin concentrations in these two studies were very similar (40 and 38 μU/ml, respectively)

Figs. 4 and 5 illustrate the relationship between insulin delivery rate and plasma glucose concentration for the fasting and the glucose infusion studies, respectively. This relationship was also evaluated with insulin delivery rate expressed either per kilogram of body weight or per square meter of body surface area. Neither transformation altered the conclusions which may be drawn. In general, the higher the plasma glucose concentration, the higher was the insulin delivery rate. Subjects exhibiting the most severe degrees of hyperglycemia, however, tended to have low insulin delivery rates. There was considerable variation in insulin response among subjects with es-

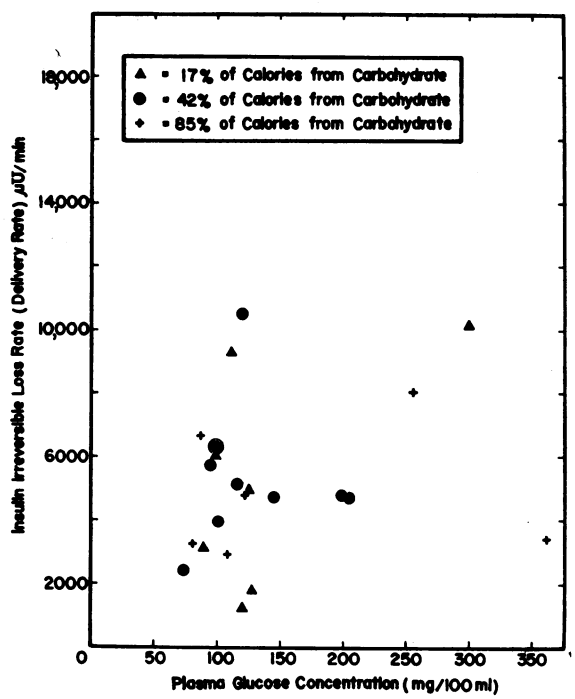

FIGURE 4 Relationship between plasma glucose concentration and insulin delivery rate during fasting. Circled characters refer to studies performed using continuous infusion of isotope technique.

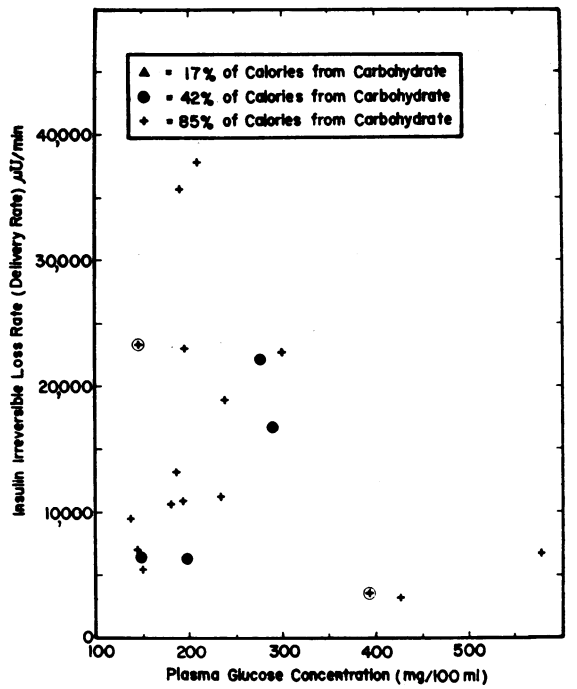

FIGURE 5 Relationship between plasma glucose concentration and insulin delivery rate during continuous infusion of glucose. Circled characters refer to studies performed using continuous infusion of isotope technique.

sentially identical plasma glucose concentrations. Thus, in the fasting studies, glucose concentrations approximating 100 mg/100 ml were associated with insulin delivery rates ranging from 2940 to 9282 μU/min. Similarly, in the glucose infusion studies, glucose concentrations of approxi-

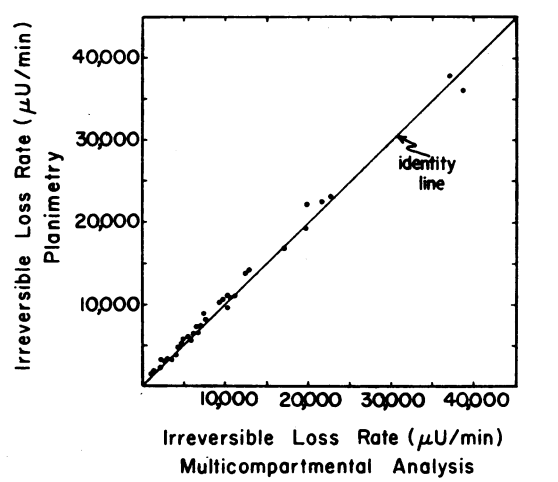

FIGURE 6 Comparison between irreversible loss rate determined by planimetry and by multicompartmental analysis.

mately 150 mg/100 ml were associated with insulin delivery rates ranging from 5616 to 23,332 μU/min, and glucose concentrations very close to 200 mg/100 ml were associated with insulin delivery rates ranging from 6278 to 37,818 μU/min. Thus, different subjects deliver widely different amounts of insulin to the general circulation in order to maintain similar steady-state plasma glucose concentrations.

The data of Figs. 2–5 were obtained from equation 5 with the area obtained by the method of planimetry. Fig. 6 reveals that the data could as well have been plotted with the loss rate calculated by the technique of multicompartmental analysis, since the two methods gave almost identical results.

DISCUSSION

Plasma insulin specific activity time curves following single injection of insulin-^{131}I are highly curvilinear on semilog paper, indicating that insulin does not distribute throughout a single well-mixed pool turning over with a single rate constant. In this paper we have used a technique for determining the rate at which insulin is irreversibly lost from the plasma insulin pool based on the measurement of the area under the specific activity time curve plotted on ordinary coordinates. The mathematical basis of this technique has been presented, and it has been shown that measurement of irreversible loss rate of insulin does not require a knowledge of the number of pools throughout which insulin distributes, nor their manner of interaction. It would appear, therefore, that there is little justification for the use of the extrapolation technique inasmuch as the technique described in this paper is equally simple to apply and does not involve dubious assumptions about the applicability to multicompartmental systems of methods suited only to single compartments.

Although the theoretical basis of the planimetry method is sound, its practical application is subject to error arising principally from the extrapolation of the tail end of the curve and hence, this technique cannot be used indiscriminately. Estimation of irreversible loss rate by the techniques of multicompartmental analysis requires that one assume that there are no components of turnover slower than that revealed by the slowest exponential implicit in the actual data. If there were, an area of undetermined magnitude would be added to the area under the specific activity time curve. Unfortunately, there is no known method of validating this assumption although the longer the time period of data collection the less likely a slow component of turnover may be missed. There was excellent agreement between the planimetry calculations and the computer calculations (Fig. 6). This indicates that the extrapolations of the tail end of the curves which were made by eye agreed with the computer estimate of the slowest exponential contained within the data. We also feel that the results of the continuous infusion of isotope studies tend to confirm the planimetry results, although, inasmuch as we have only three of this type of experiment, we do not rest heavily on this point.

Determination of the irreversible loss rate of insulin does not constitute a complete description of the multicompartmental system which must exist for insulin metabolism. Insulin is secreted into the portal circulation, and a significant portion of it is removed by the liver (19), and thus never enters the plasma compartment. Therefore, knowledge of irreversible loss rate from plasma does not directly provide information about insulin secretion rate or hepatic clearance of insulin. Estimates for these parameters can be arrived at by the study of various models of insulin metabolism with techniques of multicompartmental analysis (18). Unlike irreversible loss rate, however, these estimates are dependent upon model formulation and require independent experimental verification. We are currently engaged in efforts to establish a more complete model of insulin metabolism in man.

Although the analyses which we can currently make are somewhat limited, they nonetheless provide useful physiological information. Changes in plasma insulin concentration were associated with corresponding changes in the rate of delivery of newly secreted insulin to the general circulation, and not with changes in the opposite direction of insulin removal. Furthermore, the relationship between concentration and delivery rate was linear over a wide range of insulin concentration. This linearity implies lack of saturability of plasma insulin removal mechanisms, a desirable feature of a control system in which rapid adjustments of plasma concentration are necessary. Finally, these

studies reveal that widely different insulin delivery rates may be associated with essentially identical plasma glucose concentrations. In this regard, the response to glucose infusion of patients with normal or nearly normal oral glucose tolerance (W.W., W.G., G.L., F.B., and W.K.) is of particular interest. In such subjects, hepatic glucose output is known to be markedly decreased by glucose infusion (20), and it is therefore likely that endogenous glucose entry into the circulation during these studies was minimal. Under these circumstances, since plasma glucose concentration was constant, glucose uptake must have closely approximated the rate of glucose infusion. These five patients attained essentially identical steady-state plasma glucose concentrations (150 mg/100 ml) in response to a continuous glucose infusion of 7 mg/kg of body weight per min (Fig. 5). Thus, although these patients were disposing of glucose at essentially similarly rates and at comparable levels of plasma glucose concentration, they nevertheless delivered markedly different amounts of insulin to the general circulation. Unless hepatic insulin clearance simultaneously varied in the opposite direction, these results suggest that there are major differences between individuals in the amount of insulin apparently required to establish similar rates of glucose uptake at essentially the same level of plasma glucose concentration.

Although the results of this study imply that it is permissible to interpret plasma insulin concentration as directly reflecting insulin delivery rate, this correlation applies only during steady-state conditions. When a change in delivery rate occurs, a finite period of time must elapse before the total body insulin pool can expand or contract to the new steady-state level. This "lag period" introduces great complexity into the analysis of non-steady-state insulin concentration curves such as occur during a glucose tolerance test. For example, when insulin delivery is increased in response to a hyperglycemic stimulus, a fall in blood sugar is brought about so that the stimulus to increased insulin delivery is not sustained. Therefore, the maximum insulin concentration which is achieved may well be less than that which would have resulted had the increased delivery rate persisted until a new steady-state insulin concentration had become established. Under these circumstances, the change in insulin concentration does not adequately reflect the change in insulin delivery rate which produced it.

Interpretation of these studies is contingent upon the usual assumptions that the insulin degradation system does not distinguish between the isotopic tracer chosen and the native unlabeled material. In particular, it is required: (*a*) that labeled insulin be biologically active; (*b*) that loss of the radioactive iodine label from the insulin molecule not occur except when the insulin molecule is itself degraded; and (*c*) that the rates of degradation of labeled and unlabeled and of human and beef insulin be similar. Recently, Arquilla, Ooms, and Finn (21) have suggested that a significant portion of insulin ^{125}I, even when only lightly iodinated, is biologically inactive in the rat epididymal fat pad assay. However, there is considerable evidence (22, 23) that insulin-^{131}I retains close to 100% hypoglycemic potency at levels of iodination comparable to those used by Arquilla, et al. In particular, Izzo, Ronbone, Izzo and Bale (22) have recently studied this matter thoroughly using three bioassay systems (mouse convulsion test, rat epididymal fat pad, and rat hemidiaphragm), and have found that when insulin is iodinated with ^{131}I to a level of one iodine atom per molecule of insulin (6000 mol wt) 100% biological activity is preserved. The insulin-^{131}I used in these experiments contained considerably less than one atom of iodine per molecule of insulin.

Preserved hormonal function, however, does not rule out the possibility that some more subtle change in the insulin molecule could exist which alters its degradation rate. Direct deiodination of insulin by in vitro systems has not been demonstrated (23, 24). Furthermore, Tomizawa, Nutley, Narahara, and Williams (24) have shown that when insulin-^{131}I is incubated with rat liver homogenates, loss of trichloracetic acid (TCA)-precipitable radioactivity and of hypoglycemic potency of the incubation mixture proceed at the same rate. Elgee and Williams (25) have shown that preloading with insulin diminished the rate of insulin-^{131}I degradation in vivo, and that the extent of inhibition was the same regardless of whether the preloading was accomplished with labeled or unlabeled insulin, implying that both types of insulin competed equally well for the degradation system. Beck, Roberts, Blanken-

baker, and King (26) have shown that the distribution and rates of accumulation of labeled and unlabeled insulin in various organs and subcellular fractions were similar. Finally, Samols and Ryder (27) have shown that hepatic clearance of exogeneously administered beef insulin was similar to the clearance of endogenous human insulin in human subjects. On the basis of these studies, it is reasonable to conclude that deiodination of insulin does not proceed independently of complete degradation of the insulin molecule itself, and that the rates of degradation of lightly iodine-labeled and unlabeled insulin are similar. More evidence regarding possible differences in handling of insulin derived from different species, however, would be desirable.

ACKNOWLEDGMENTS

We would like to thank Mrs. Nancy Ballot, Mrs. Jorene Moore, and Mr. William Smith for their excellent technical assistance. We would also like to thank Mrs. Barbara Stern for valuable assistance with the computer analyses and for planimetering the insulin specific activity time curves. We would also like to thank Dr. Nome Baker for his guiding discussions during the early phases of this work. Finally, we are grateful to the staff of the Stanford General Clinical Research Center for their excellent assistance in patient care and diet preparation.

This work was supported in part by the U. S. Public Health Service Grant HE 08506 from the National Institutes of Health, K3-HE 6003 from the Career Development Review Branch, and Fr-70 from the General Clinical Research Centers Branch, Division of Research Facilities and Resources.

REFERENCES

1. Yalow, R. S., and S. A. Berson. 1960. Immunoassay of endogenous plasma insulin in man. *J. Clin. Invest.* **39**: 1157.
2. Berson, S. A., R. S. Yalow, A. Bauman, M. A. Rothschild, and K. Newerly. 1956. Insulin-I^{131} metabolism in human subjects: demonstration of insulin binding globulin in the circulation of insulin treated subjects. *J. Clin. Invest.* **35**: 170.
3. Welsh, G. W., E. D. Henley, R. H. Williams, and R. W. Cox. 1956. Insulin I^{131} metabolism in man. Plasma-binding distribution and degradation. *Am. J. Med.* **21**: 324.
4. O'Brien, J. P., and A. R. Sharpe, Jr. 1967. The influence of renal disease on the insulin I^{131} disappearance curve in man. *Metab. Clin. Exptl.* **16**: 76.
5. Bolinger, R. E., and H. J. Grady. 1958. The plasma insulin-I^{131} in diabetic patients. *Ann. Intern. Med.* **48**: 753.
6. Palmer, D. L., and R. E. Bolinger. 1961. Effect of nephrectomy and splanchnicectomy on plasma disappearance of labeled insulin in the rabbit. *Proc. Soc. Exptl. Biol. Med.* **107**: 809.
7. Bolinger, R. E., and R. R. Stephens. 1964. Comparison of disappearance from plasma of insulin I^{131} and of insulin-like activity. *Proc. Soc. Exptl. Biol. Med.* **116**: 812.
8. Ricketts, H. T., H. L. Wildberger, and L. Regut. 1963. The role of the kidney in the disposal of insulin in rats. *Diabetes.* **12**: 155.
9. Scott, G. W., T. E. Prout, J. A. Weaver, and S. P. Asper. 1958. A comparison of the behavior of insulin and insulin labeled with I^{131} in serum. *Diabetes.* **7**: 38.
10. Zaharko, D. S., L. V. Beck, and R. Blankenbaker. 1966. Role of the kidney in the disposal of radioiodinated and non radioiodinated insulin in dogs. *Diabetes.* **15**: 680.
11. 1964. Plasma glucose procedure N-9. *In* Auto-Analyzer Manual. Technicon Instruments Corporation, Chauncey, N. Y.
12. Grodsky, G. M., and P. H. Forsham. 1960. An immunochemical assay of total extractable insulin in man. *J. Clin. Invest.* **39**: 1070.
13. Grodsky, G. M., and P. H. Forsham. 1961. Comparative binding of beef and human insulin to insulin antibodies produced in man and guinea pigs. *J. Clin. Invest.* **40**: 799.
14. Hales, C. N., and P. J. Randle. 1963. Immunoassay of insulin with insulin-antibody precipitate. *Biochem. J.* **88**: 137.
15. Baker, N., R. A. Shipley, R. E. Clark, and G. E. Incefy. 1959. C^{14} studies in carbohydrate metabolism: glucose pool size and rate of turnover in the normal rat. *Am. J. Physiol.* **196**: 245.
16. Tait, J. F. 1963. Review: The use of isotopic steroids for the measurement of production rates *in vivo*. *J. Clin. Endocrinol. Metab.* **23**: 1285.
17. Zierler, K. L. 1962. Circulation times and the theory of indicator-dilution methods for determining blood flow and volume. *In* Handbook of Physiology. W. F. Hamilton and P. Dow, editors. American Physiological Society, Washington, D. C. **1**: 585.
18. Berman, M., E. Shahn, and M. F. Weiss. 1962. The routine fitting of kinetic data to models: a mathematical formalism for digital computers. *Biophys. J.* **2**: 275.
19. Barrett, P. V. D., P. D. Berk, M. Menken, and N. I. Berlin. 1968. Bilirubin turnover studies in normal and pathologic states using bilirubin-^{14}C. *Ann. Intern. Med.* **68**: 355.
20. Madison, L. L., D. Mebane, F. Lecocq, and B. Combes. 1963. Physiological significance of the secretion of endogenous insulin into the portal circulation. V. The quantitative importance of the liver in the disposition of glucose loads. *Diabetes.* **12**: 8.
21. Arquilla, E. R., H. Ooms, and J. Finn. 1966. Review of current research: genetic differences of combining sites of insulin antibodies and importance of C-terminal portion of the A chain to biological and immunological activity of insulin. *Diabetologia.* **2**: 1.

22. Izzo, J. L., A. Roncone, J. J. Izzo, and W. F. Bale. 1964. Relationship between degree of iodination of insulin and its biological, electrophoretic, and immunochemical properties. *J. Biol. Chem.* **239**: 3749.
23. Lee, N. D. 1957. Studies on insulin labeled with I^{131}. *Ann. N. Y. Acad. Sci.* **70**: 94.
24. Tomizawa, H. H., M. L. Nutley, H. T. Narahara, and R. H. Williams. 1955. The mode of inactivation of insulin by rat liver extracts. *J. Biol. Chem.* **214**: 285.
25. Elgee, N. J., and R. H. Williams. 1955. Specificity of insulin degradation reaction. *Diabetes.* **4**: 87.
26. Beck, L. V., N. Roberts, R. Blankenbaker, and C. King. 1966. On insulin immunologic activity in livers and kidneys of mice injected with beef insulin. *Proc. Soc. Exptl. Biol. Med.* **122**: 768.
27. Samols, E., and J. A. Ryder. 1961. Studies on tissue uptake of insulin in man using a differential immunoassay for endogenous and exogenous insulin. *J. Clin. Invest.* **40**: 2092.