

Derivation of a Three Compartment Model Describing Disappearance of Plasma Insulin-¹³¹I in Man

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ABSTRACT Insulin-¹³¹I was administered intravenously to normal subjects, to patients with maturity-onset diabetes and normal renal function, and to non-diabetic patients with renal failure. The ensuing plasma disappearance curves of immunoprecipitable radioactive insulin were determined, and these data were analyzed in a variety of ways. Firstly, fractional irreversible loss rates of insulin from plasma were calculated and found to be greatly diminished in patients with renal failure ($t_{1/2} = 39$ min), as compared with normal ($t_{1/2} = 15$ min) and diabetic subjects ($t_{1/2} = 12$ min). Secondly, plasma insulin-¹³¹I disappearance curves were resolved into sums of three exponentials by the method of "peeling," and values for the resultant three slopes and half-lives were determined. Patients with normal renal function had similar values for all parameters, while those patients with renal failure were differentiated on the basis of the slope of the last component, with a prolongation of its half-life to 275 min (approximately twice normal). Finally, a three pool model was formulated to describe the kinetics of plasma insulin disappearance in man, representing plasma (pool 1), interstitial fluid (pool 2), and all tissues in which insulin is utilized and degraded (pool 3). The proposed model adequately describing the disappearance curves of insulin-¹³¹I observed in all patients indicated that volumes (per cent body weight) of pool 1 (4.04) and pool 2 (10.11), calculated on the basis of the model and the experimental data, corresponded closely to estimates of plasma and interstitial fluid vol-

umes obtained by independent means. It also demonstrated that patients with renal failure were characterized by a decreased removal rate of insulin from pool 3 and an increased recycling rate of insulin from pool 3 to pool 2. It is concluded that the proposed model represents a reasonable description of the kinetics of insulin distribution and degradation, and that its use provides quantitative insights into the physiology of insulin metabolism.

INTRODUCTION

In a recent paper we demonstrated that disappearance of insulin-¹³¹I from plasma of man clearly reflected a non-first order process (1). The potential errors resulting from the use of first order concepts to analyze such situations were pointed out, and alternative methods were described which did permit estimation of the rate at which insulin was being delivered into the general circulation. This analysis represented the most primitive approach to a description of the multicompartmental system involved in insulin distribution and degradation in man. In an effort to begin construction of a more sophisticated model of insulin kinetics, we have extended our studies of insulin-¹³¹I plasma disappearance to include patients with terminal renal failure. These patients were selected because there is considerable evidence suggesting that the kidney plays an important role in insulin degradation and excretion (2) and, consequently, plasma insulin-¹³¹I disappearance curves of such patients might be reasonably expected to differ substantially from those we had previously described in normal and maturity-onset diabetic subjects. We hoped that comparison of results in patients with normal renal function with similar studies of patients with essentially no renal function would aid in our initial attempts at model building. The results indicated that in-

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TABLE I
Clinical Characteristics

	Number of patients	Age*	Fasting plasma glucose‡	Serum creatinine‡
		yr	mg/100 ml	mg/100 ml
Normal	6	53.0 ± 7.1	82.2 ± 6.3	1.03 ± 0.09
Diabetic	8	55.6 ± 8.7	179.9 ± 43.9	1.11 ± 0.06
Renal failure	4	29.3 ± 10.7	104.0 ± 4.1	18.42 ± 2.3

* Mean ± SD.

‡ Mean ± SE.

sulin-¹²⁵I disappearance curves from plasma of patients with terminal renal failure were significantly different from either diabetic or normal subjects with normal renal function. Further analysis of the results from all subjects led to the formulation of a three pool model of insulin kinetics in man. Although there were significant differences in the rate constants between various pools in the three population groups, the three pool model adequately described the disappearance curves of insulin-¹²⁵I observed in all patients. In this paper we shall describe the manner in which the model was derived, as well as some attempts to validate its appropriateness.

METHODS

Experimental subjects. 14 patients with normal renal function were studied. Six had normal oral glucose tolerance tests (3), while the other eight had maturity-onset diabetes mellitus. None of the patients with diabetes had ever received insulin. Four subjects in end-stage renal failure awaiting renal transplantation were studied twice, once before nephrectomy and again, in the anephric state, immediately before transplantation. These subjects had chronic parenchymal renal disease without evidence of systemic disease or diabetes. Table I summarizes the characteristics of the three population groups.

Experimental protocol. All patients received over 300 g of carbohydrate daily for at least 1 wk before measurement of plasma insulin-¹²⁵I disappearance. Studies were performed after a 15 hr fast. Insulin-¹²⁵I (0.75 μc/kg body weight)¹ was given by rapid intravenous injection and blood obtained via an indwelling polyethylene catheter. Samples were obtained 2, 4, 6, 10, 15, 20, and 30 min after injection of the tracer and then every 15 min for the next 2½ hr.

Technical procedures. Blood was drawn into tubes containing ethylenediaminetetraacetate (EDTA). Plasma, promptly separated in a refrigerated centrifuge, was immediately frozen in acetone-dry ice. Disappearance of immunoprecipitable radioactivity from plasma was determined the following day by the method of Grodsky and Forsham (4, 5), 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, recovery of immunoprecipitable radioactivity was greater than 95% over a wide range of "cold" porcine insulin concentrations (10–1000 μU/ml). The total dose of immunoprecipitable radioactivity administered to each subject was determined by applying the above method to aliquots of injected material saved from each experiment.

Computational methods. The data, expressed in counts per minute per milliliter of plasma, described a nonlinear curve on semilog paper. The curve was resolved into a sum of exponentials by the method of "peeling" (6). The results of peeling were used as initial estimates to a nonlinear least square program developed by Powell (7) and adapted for use on the IBM 360/50 on line computer. We obtained a fitted sum of exponentials which were used to compute fractional turnover rates on the same on line computer. These fractional rates were then used as initial estimates to the SAAM program of Berman and Weiss (8). This program obtained final estimates of the parameters. Final sum of squares and estimates of parameter variance were also provided which were used as indices of good fit. The final estimates of the fractional rates were used to compute a simulated curve of plasma insulin disappearance, which was visually displayed with the data on a cathode ray tube at the Stanford Medical School computer facility. Some of the figures in this text are photographic reproductions of the display.

A stepwise discriminant analysis (9) was applied to parameters obtained from the Berman program. The analysis was performed on an IBM 360/67 computer in which the biomedical program library is stored.

RESULTS

Fig. 1 shows three typical plasma insulin-¹²⁵I disappearance curves obtained, respectively, from subjects with normal glucose tolerance and renal function, abnormal glucose tolerance and normal renal function, and those with abnormal renal function. These curves are clearly nonlinear on semilog paper and cannot represent a first order process. There are at least three ways to analyze these results in a quantitative fashion. These methods represent three levels of sophistication in terms of kinetic analysis and are capable of yielding increasingly more detailed information relevant to the multi-compartmental system being investigated.

The simplest approach is to plot the disappearance curve of plasma immunoprecipitable radioactivity on graph paper with ordinary coordinates. The area under the circumscribed curve, estimated by planimetry, can be

TABLE II
Fractional Irreversible Loss Rate of Insulin from Plasma (λ_e)

	λ _e (1/min)*	t _{1/2} = $\frac{0.693}{\lambda_e}$ (min)*
Normal	0.048 ± 0.005	15.193 ± 1.439
Diabetic	0.060 ± 0.002	11.627 ± 0.285
Renal failure	0.020 ± 0.003	39.210 ± 6.158

* Mean ± SE.

¹ Bovine-insulin-¹²⁵I, approximately 4 mc/mg, Abbott Laboratory, North Chicago, Ill.

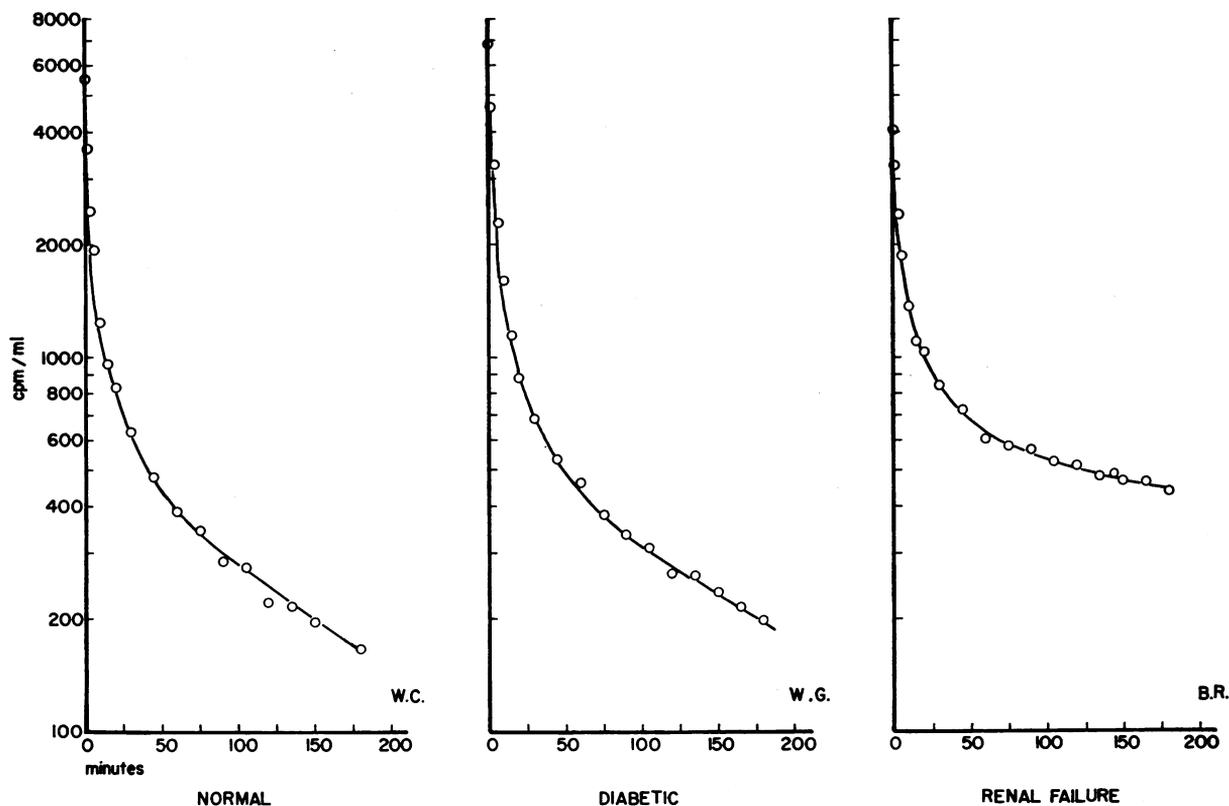


FIGURE 1 Typical insulin-¹²⁵I plasma disappearance curves for each patient group.

divided into the dose of administered tracer, and this answer, with appropriate conditions, represents fractional irreversible loss rate of insulin from plasma. (The mathematical basis for this method has been discussed in detail in a recent paper from our laboratory [1].) Fractional irreversible loss rates from plasma for the three patient groups have been estimated by this method, and the results appear in Table II. An estimate of the half-life of this fractional rate can be determined, and this is also shown in Table II. It can be seen that the fractional irreversible loss rate of plasma insulin and the half-life of this loss are essentially similar in the two groups of patients with normal renal function, whereas patients with terminal renal failure had greatly decreased fractional rates of loss and increased half-lives. (Patients with renal failure had essentially identical plasma insulin-¹²⁵I disappearance curves before and after nephrectomy, and data from both experiments for each patient are used in this and all subsequent analyses.)

Although this method provides valid quantitative information which clearly distinguishes patients with renal failure from those with normal renal function, it does not begin to describe the multicompartmental system which must exist for insulin metabolism. A second and more complicated quantitative approach is based

upon resolution of plasma insulin-¹²⁵I disappearance curves into various numbers of components by the method of peeling. Fig. 2 A displays one of the curves of Fig. 1 (W. C.) in terms of two components. The mathematical equation describing this latter set is given by: plasma insulin-¹²⁵I (cpm/ml) = $h_1 e^{-g_1 t} + h_2 e^{-g_2 t}$, where g_1 and h_1 are the slope and intercept, respectively, of the first component and g_2 and h_2 the equivalent parameters for the second component. The same data in Fig. 1 can also be resolved by the peeling procedure into three components. This result is shown in Fig. 2 B. The mathematical description for this set is plasma insulin-¹²⁵I (cpm/ml) = $h_1 e^{-g_1 t} + h_2 e^{-g_2 t} + h_3 e^{-g_3 t}$, whereas in the previous case, g_i and h_i are, respectively, the slope and intercept of the i^{th} component, and i is either the index 1, 2, or 3. Further resolution of these curves is not possible, since a fourth component is not discernible by eye nor can it be obtained by the peeling procedure. Solution of these equations provides data which are useful in a variety of ways. For example, values for slopes and half-lives of the components can be obtained and patients compared on this basis. Results of such an analysis of the three component case are seen in Table III. Patients with normal renal function had comparable values for half-lives, while patients with renal

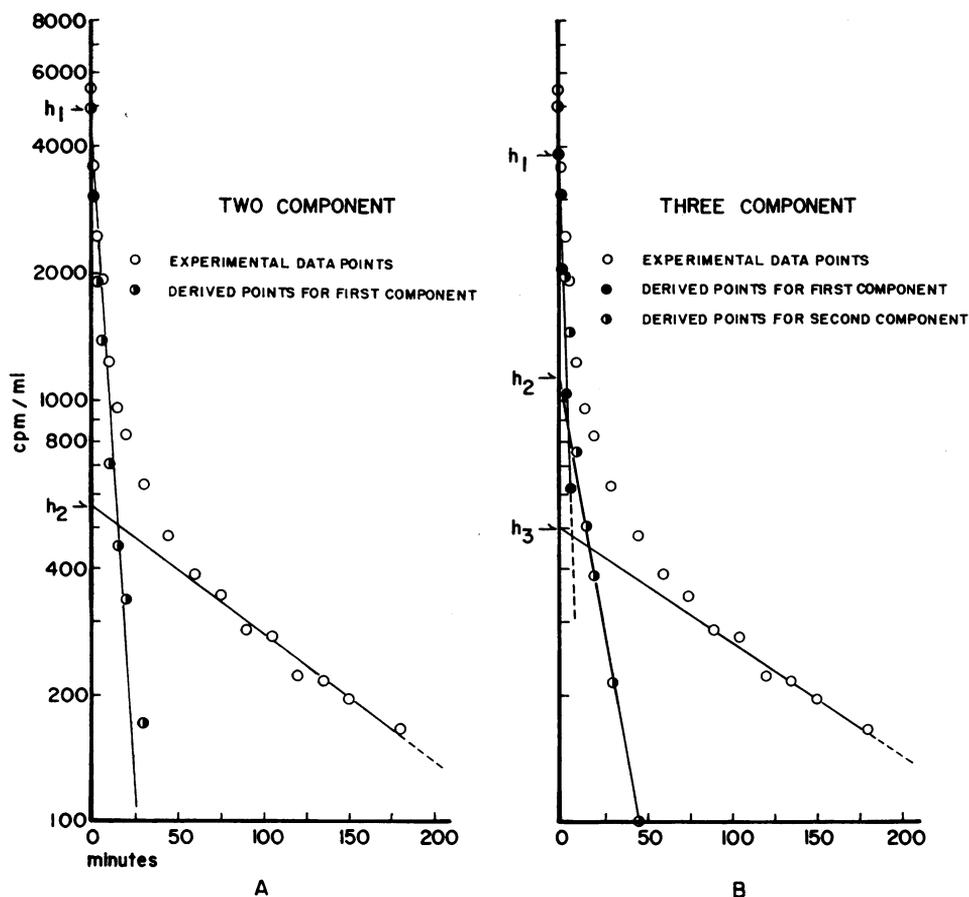


FIGURE 2 An insulin-¹²⁵I plasma disappearance curve (subject W. C.) resolved into two and three components.

failure differed from the other two groups, with the main difference being the half-life of the last component. Additionally, a comparison between the "goodness of fit" of the two vs. the three component case can be made and a decision reached as to which analysis is most appropriate. Although this procedure may indicate which case will generate the most meaningful data, it is apparent that the results of this analysis are not necessarily relevant to any specific biological system. In order to accomplish this it is necessary to postulate a multicompartmental model, and the data derived from peeling is used to provide initial estimates in this procedure as described in the following section.

A multicompartmental model is formulated in terms of the minimal number of components available; therefore, resolution of the plasma insulin-¹²⁵I disappearance curves as the sum of two exponentials (Fig. 2 A) indicated that a two compartment model might be adequate (10). The model depicted in Fig. 3 seemed reasonable as a first approximation. Fig. 3 depicts a mechanism in which insulin travels from plasma (compartment 1) to

an extra-plasma pool (compartment 2) which is the site of activity and degradation of insulin. The parameters λ_{1j} are fractional turnover rates, and the subscripts denote passage from compartment j to compartment i , i.e., λ_{21} = fractional rate of plasma insulin leaving plasma and entering compartment 2, λ_{22} = fractional rate of insulin returning to plasma from compartment 2,

TABLE III
Half Life of Component Slopes (t_1)*

Three component case		
First component‡	Second component‡	Third component‡
2.411 ± 0.192	14.114 ± 0.623	132.80 ± 11.88
1.875 ± 0.260	8.281 ± 0.497	101.18 ± 7.40
1.557 ± 0.217	10.400 ± 1.272	275.54 ± 31.30

* t_1 was obtained from the component slopes using the formula $t_1 = \frac{0.693}{\text{slope}}$ and is expressed in minutes.

‡ Mean ± SE.

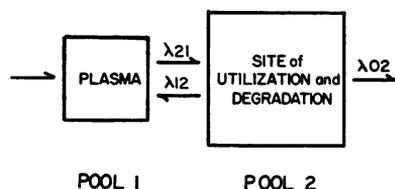


FIGURE 3 A two compartment model describing insulin-¹²⁵I plasma disappearance, in which λ_{1i} are fractional rates of transfer.

and λ_{02} is the fractional rate of insulin irreversibly leaving the site of degradation. The model in Fig. 3 is based on the assumption that insulin enters plasma from the pancreas, leaves plasma, can return unchanged, and is utilized and degraded in the extra-plasma pool.

The appropriateness of this model was tested in the following manner. Estimates of the parameters ($h_i, g_i, i = 1, 2$) describing a sum of two exponentials were initially obtained by the process of peeling. The values for these parameters were refined by the fitting procedure developed by Powell (7). The refined h_i and g_i were used to compute initial estimates of λ_{1i} by the following formulae:

$$\lambda_{21} = g_2 - \frac{h_1}{h_1 + h_2}(g_2 - g_1)$$

$$\lambda_{02} = g_1 g_2 \div \left[g_2 - \frac{h_1}{h_1 + h_2}(g_2 - g_1) \right]$$

$$\lambda_{12} = g_1 + \frac{h_1}{h_1 + h_2}(g_2 - g_1) - \lambda_{02}$$

The final estimates obtained from the SAAM program (8) (see computational methods) were used in a simulation procedure to see how well they predicted the actual experimental data. The results of this simulation are seen in Fig. 4. Although the data seem to be satisfactorily reproduced by the theoretical curve during the first 50 min, subsequent data points are seen to divert significantly from the predicted curve. This inadequate simulation of the experimental data was true for all

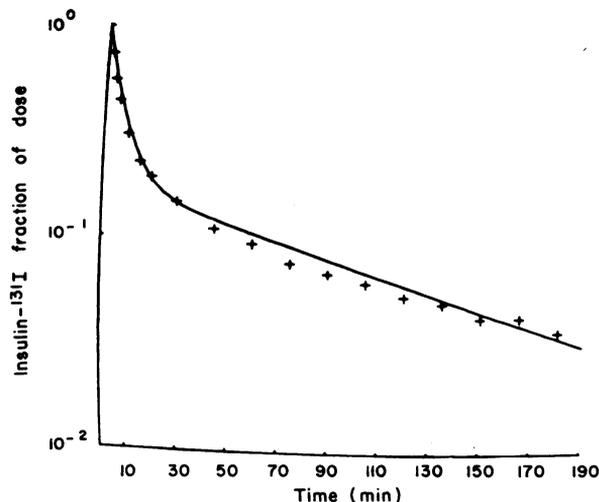


FIGURE 4 Simulation of a normal patient's disappearance obtained from the two pool model. The + are the data points, and the continuous curve is the calculated curve describing the data. The ordinate is expressed in fraction of dose and the abscissa in minutes. This is a photograph of the data and simulated curve as displayed on a cathode ray tube.

patients, and a two pool model was rejected as representing the minimal model necessary to explain the data.

A three pool model was then formulated which is illustrated in Fig. 5. The first pool in this model is plasma. The second pool, exchanging with plasma, is the interstitial fluid compartment, and the third pool is the compartment in which insulin is utilized and degraded, and from which it is irreversibly removed. The fractional transfer rates are defined in the same manner as in the two pool model. Analogous to λ_{02} in the previous model, λ_{03} is the fractional irreversible loss rate of insulin leaving the site of degradation. To initiate the procedure in the fitting program, we provided estimates of the λ_{1i} to the computer. These estimates were obtained using the methodology described by Skinner, Clark, Baker, and Shipley (11). A summary of the final estimates of the parameters λ_{1i} obtained from the SAAM program is given in Table IV.

TABLE IV
Summary of Model Parameters

	λ_{21}	λ_{12}	λ_{22}	λ_{23}	λ_{03}
I	0.225 ± 0.053	0.065 ± 0.014	0.047 ± 0.007	0.014 ± 0.003	0.008 ± 0.002
II	0.283 ± 0.054	0.091 ± 0.023	0.077 ± 0.012	0.021 ± 0.003	0.010 ± 0.002
III	0.338 ± 0.134	0.136 ± 0.047	0.064 ± 0.024	0.028 ± 0.008	0.005 ± 0.002

All values are expressed as means ± SD.

I = Normal glucose tolerance tests (GTT) and normal renal function.

II = Abnormal GTT and normal renal function.

III = Normal GTT and terminal renal failure.

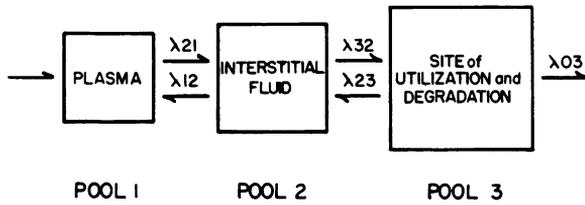


FIGURE 5 A three compartment model describing insulin-¹³¹I plasma disappearance.

The simulation obtained from this model is shown in Fig. 6, and again is representative of the results for all patients. The curvature of the data from 50 to 180 min is more closely approximated in the three pool model than in the two pool model (Fig. 4). The weighted sum of squares of residuals, provided in the last iteration of the nonlinear least square portion of the Berman program, are used to numerically judge the closeness of fit. Fig. 7 shows that the mean sum of squares obtained from the data of all subjects for the two compartment model is approximately seven times the value obtained for the three pool model, a finding thus indicating the enhanced fit provided by the latter model. The program also provides fractional deviations of the parameters which are calculated in the final iteration. The mean fractional deviation (\pm SE) of all parameters for all patients in the three pool model is 0.21 ± 0.019 . This small figure further indicates the compatibility of the model to the data.

Another way to evaluate the appropriateness of a given model is to see whether or not the volume of postulated pools, calculated on the basis of the model and experimental data, correspond to the sizes of anatomical pools derived from direct measurement. In the case of the postulated three pool model we can generate estimates for pools 1 (plasma) and 2 (interstitial fluid). The sizes of these two pools, as well as both their sum and the ratio between them, can then be compared with independent estimates of these parameters.

In order to compute the size of pool 1 we used the following formula:

Plasma volume = $\frac{\text{dose}}{h_1 + h_2 + h_3}$ and obtained a value of 4.04% of body weight as the average plasma volume (V_1) for all subjects. The volume, V_2 , of pool 2 was derived from the following equation:

$$V_2/V_1 = \frac{\lambda_{21}\lambda_{33}}{\lambda_{22}\lambda_{33} - \lambda_{23}\lambda_{32}}$$

where $\lambda_{22} = \lambda_{12} + \lambda_{32}$ and $\lambda_{33} = \lambda_{23} + \lambda_{03}$.

This equation is based upon the assumption of a steady and uniform concentration of insulin throughout the ex-

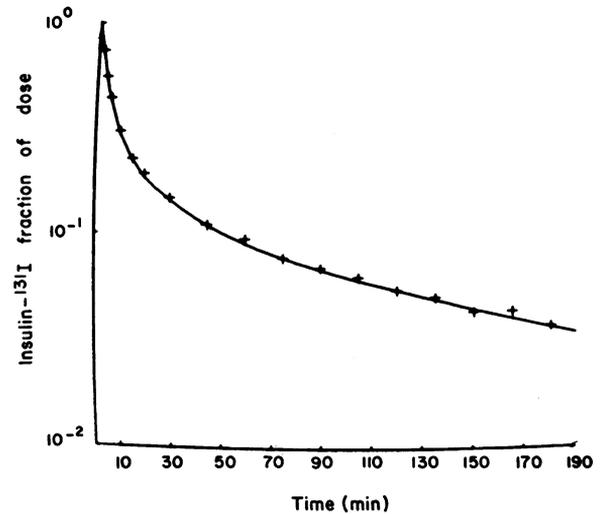


FIGURE 6 Simulation obtained from the three pool model. The + are the data points, and the continuous curve is the calculated curve describing the data. The ordinate is expressed in fraction of dose and the abscissa in minutes. This is a photograph of the data and simulated curve as displayed on a cathode ray tube.

tracellular space (12). The value of the ratio V_2/V_1 for patients was 2.51 ± 0.46 (Mean \pm SD). Thus, the volume of interstitial fluid was calculated to be equal to 10.14% of body weight ($2.51 \times 4.04\%$) and total extracellular fluid volume to be equal to 14.18% of body weight ($4.04\% + 10.14\%$). The results of these calculations are compared to similar data obtained by direct measurement in Table V and can be seen to closely correspond to the mean of previous estimates of several

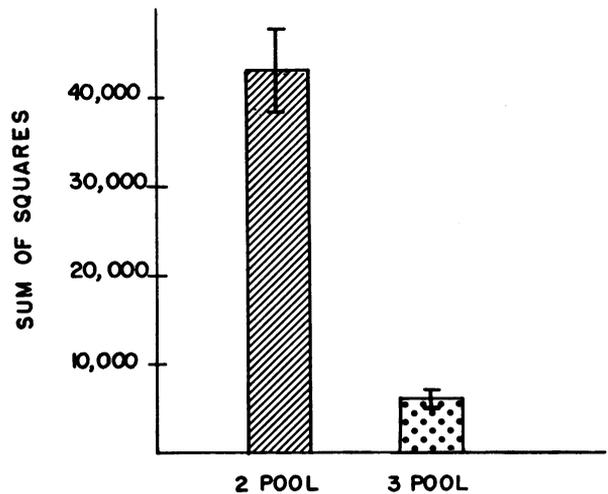


FIGURE 7 A comparison of the sum of squares (Mean \pm SE) obtained in the output of the SAAM program, indicating the better fit with use of a three pool model.

TABLE V
Comparison of Estimates of Plasma and Interstitial
Fluid Volumes

	Plasma volume (PV)	Interstitial fluid volume (IFV)	Extracellular fluid volume	IFV/PV
	<i>% body weight</i>	<i>% body weight</i>	<i>% body weight</i>	
Others	4.4*	11.4‡	15.8§	2.59
Current study	4.04	10.14	14.8	2.51

* Plasma volume based on the measurement of the volume of distribution of radioiodinated serum albumin (17); mean of 25 patients.

‡ 11.4 = extracellular fluid volume (ECF) – plasma volume = 15.8 – 4.4.

§ ECF volumes based on the measurement of the volume of distribution of inulin (12–16); mean of 36 patients.

investigators (13–18). The close approximation of these estimates of pool size provides further support for the postulated model.

The fractional turnover rates of the three clinical groups were compared by stepwise discriminant analysis in an effort to identify which parameter(s) might most distinguish differences between the groups. These results are seen in Table VI, in which the comparisons are listed in decreasing order of discrimination of *F* values. This analysis shows that the most discriminating fractional turnover rates between subjects with normal renal function (both with normal or abnormal glucose tolerance), and those with end-stage renal failure were λ_{03} and λ_{23} . λ_{03} is the fractional irreversible loss of insulin from pool 3, and λ_{23} is the fractional recycling rate from the same pool back into interstitial fluid. Finally, since λ_{03} and λ_{23} are exit rates from the same compartment, we might expect these parameters to relate to each other. A correlation matrix obtained in the output of the discriminant analysis program does suggest such a relationship for λ_{03} and λ_{23} , with a correlation coefficient of 0.71 when normal subjects were compared with those with renal failure, and 0.76 when subjects with abnormal glucose tolerance tests were compared with subjects in renal failure.

DISCUSSION

Plasma disappearance curves of insulin-¹²⁵I are curvilinear when graphed on semilog paper, indicating that a multicompartmental system exists for removal of insulin from plasma of man. In this paper we have applied the techniques of multicompartmental analysis in order to gain additional understanding of the kinetics of insulin distribution and degradation. Our results have led us to propose a three pool model as the minimal model necessary to explain the observed disappearance curves

TABLE VI
Summary Table for Stepwise Discriminant Analysis

Group comparisons*	Step No.	Parameter entered	<i>F</i> value	Number of variables included
I vs. II	1	λ_{22}	37.9568	1
	2	λ_{23}	9.1817	2
	3	λ_{21}	1.1801	3
	4	λ_{03}	0.5344	4
	5	λ_{12}	0.0638	5
I vs. III	1	λ_{23}	23.0615	1
	2	λ_{03}	36.7094	2
	3	λ_{21}	0.8685	3
	4	λ_{22}	1.5319	4
	5	λ_{12}	3.3115	5
II vs. III	1	λ_{03}	39.8882	1
	2	λ_{23}	28.5587	2
	3	λ_{22}	1.0724	3
	4	λ_{12}	5.9732	4
	5	λ_{21}	0.0000	5

*I = Normal GTT and normal renal function.

II = Abnormal GTT and normal renal function.

III = Normal GTT and terminal renal failure.

of insulin-¹²⁵I from the plasma. The first pool is thought to represent plasma, the second interstitial fluid volume, and the third all those tissues which utilize and degrade insulin. The third pool is clearly not homogenous and represents, at least, muscle, adipose tissue, kidney, and the liver.

The ability of mathematical models of this type to compute transfer rates between compartments has been demonstrated by Berman and Schoenfeld (19) and Berman, Shahn, and Weiss (20), who indicated that the properties of a multicompartmental system could be determined from isotopic disappearance from one central compartment. In order to use this approach it is necessary to assume that the insulin degradation system does not distinguish between the isotopic tracer and the native unlabeled compound. More specifically, it is essential that: (a) the labeled compound be distributed in the same manner as the endogenous compound; (b) that loss of label from the tracer compound does not occur except when the tracer compound itself is degraded; and (c) that the degradation rates of the labeled compound and the natural compound be similar. These issues, as they relate to insulin and insulin-¹²⁵I, have been considered in detail in a recent publication from our laboratory (1), and evidence currently available suggests that these assumptions are reasonable in the context of our experimental protocol.

The above considerations simply support the belief that our attempt to define a multicompartmental model describing insulin kinetics is legitimate. They do not in

any way provide validation of the particular model we have proposed. Our reason for believing that the postulated three pool model represents a reasonable first approximation of the kinetics of insulin distribution and degradation is based upon the following considerations. The model provided an excellent fit for experimental data obtained from three different patient groups. This is exemplified by the extremely low weighted sum of squares derived from consideration of all patients (Fig. 7), as well as by the fact that we were able to closely simulate the actual experimental data by use of the postulated model (Fig. 6). Furthermore, volumes of postulated pools 1 and 2, calculated on the basis of the model and the experimental data, corresponded very closely to estimates of these pool sizes obtained by the use of very different techniques (Table V).

The primary purpose of these studies was to develop a minimal model describing the distribution and degradation of insulin in man. The inclusion of three different patient populations was to aid in this process of model development and not necessarily to provide comparative data about possible differences between groups of patients. However, certain results of these comparisons seem worthy of mention. Patients with normal renal function, irrespective of their carbohydrate tolerance, appeared to behave similarly in terms of insulin kinetics. However, there was one possible exception. Discriminant analysis, as seen in Table VI, suggested that insulin in patients with maturity-onset diabetes exchanges at a more rapid rate between pools 2 (plasma) and 3 (interstitial fluid) than it does in subjects with normal glucose tolerance. Our previous work indicated that patients with modest hyperglycemia deliver a greater load of insulin to the tissue, and that this greater load of insulin is degraded at the same rate as observed in normal patients (1). Thus, the greater fractional rate of exchange noted in the current studies may be a reflection of the fact that patients with mild maturity-onset diabetes deliver more insulin to tissue sites of utilization and degradation.

Differences between patients with and without normal renal function were much more dramatic. Certain conclusions seemed to emerge, and these were independent of the method of data analysis. If one simply calculates the irreversible loss rate of insulin-²⁵¹I (Table II), it is clear that it is much slower in patients with terminal renal failure. Conversely, the half-life of this irreversible loss rate is much greater in patients with renal failure (39.2 min) than in subjects with normal renal function (15.19 and 11.63 min). If the method of curve peeling is used, three components can be identified. The plasma insulin-²⁵¹I disappearance curves of patients with terminal renal failure differed most in the slope of the terminal component. The average half-life of this

component in patients with renal failure was 275.54 min, approximately two times that of normal subjects (132.8 min). Similar results are obtained when discriminant analysis was used to evaluate the data obtained from use of the three pool model. Table VI indicates that the parameters λ_{03} and λ_{23} are the most important distinguishing features between patients with normal and abnormal renal function. Thus, the fractional rate of loss of insulin is less for the subject with terminal renal failure, whereas the per cent of insulin recycled per minute back to the interstitial fluid is greater for this group. The increased return of insulin to the systemic circulation would seem to be a reasonable consequence of the functional loss of an organ responsible for degradation of this hormone.

Our conclusion that the rate of insulin degradation is decreased in patients with renal failure is not a new one, and this possibility was first advanced to explain the apparent decrease in insulin requirements of patients with diabetic nephropathy (21). Considerable evidence has accumulated since that time indicating that the kidney plays a major role in insulin degradation, and this has been the topic of a recent excellent review article (2). However, there are only a relatively few papers dealing with the quantitative effects of kidney disease on insulin degradation (22-24), and these studies seem to suffer from certain defects. In the first place, disappearance of nonspecific trichloroacetic acid (TCA)-precipitable radioactivity has been followed, despite the fact that several authors have indicated that this method does not adequately separate intact immunoprecipitable insulin from its labeled degradation products (25-27). Secondly, the use of this technique would have the effect of accentuating the already curvilinear insulin-²⁵¹I disappearance curve and make less justifiable the use of analytical techniques appropriate only for first order processes. Our experimental approach circumvents these problems and provides results which permit quantification of the effect of kidney disease on insulin degradation rates. However, we can not provide much insight into the mechanism of this effect. The observation that fractional rate constants of all parameters remained the same after nephrectomy in patients with terminal renal failure suggests that the presence of kidneys is itself not sufficient, and that normal renal insulin degradation requires functioning kidneys. Precisely what aspect of renal function is necessary for normal insulin degradation and the role, if any, of the uremic state is the subject of our current investigations.

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