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**Research Article**

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## Human Insulin B24 (Phe → Ser)

### Secretion and Metabolic Clearance of the Abnormal Insulin in Man and in a Dog Model

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**A**bstract. We have already demonstrated that a hyperinsulinemic, diabetic subject secreted an abnormal insulin in which serine replaced phenylalanine B24 (Shoelson S., M. Fickova, M. Haneda, A. Nahum, G. Musso, E. T. Kaiser, A. H. Rubenstein, and H. Tager. 1983. *Proc. Natl. Acad. Sci. USA.* 80:7390–7394). High performance liquid chromatography analysis now shows that the circulating insulin in several other family members also consists of a mixture of the abnormal human insulin B24 (Phe → Ser) and normal human insulin in a ratio of ~9.5:1 during fasting. Although all affected subjects show fasting hyperinsulinemia, only the propositus and her father are overtly diabetic. Analysis of the serum insulin from two nondiabetic siblings revealed that normal insulin increased from ~2 to 15% of total serum insulin after the ingestion of glucose and that the proportion of the normal hormone plateaued or fell while the level of total insulin continued to rise. Animal studies involving the graded intraportal infusion of equimolar amounts of semisynthetic human [Ser<sup>B24</sup>]-insulin and normal human insulin in pancreatectomized dogs (to simulate the secretion of insulin due to oral glucose in man) also showed both a rise in the fraction of normal insulin that reached the periphery and the attainment of a brief steady state in this fraction while total insulin levels continued to rise. Separate experiments documented a decreased hepatic extraction, a decreased metabolic clearance rate, and an increased plasma half-life of human [Ser<sup>B24</sup>]-insulin within the same parameters as those determined for normal human insulin. These results form

a basis for considering (a) the differential clearance of low activity abnormal insulins and normal insulin from the circulation in vivo, and (b) the causes of hyperinsulinemia in both diabetic and nondiabetic individuals who secrete abnormal human insulins.

#### Introduction

Three individuals from separate families are now known to secrete distinct, abnormal insulins that result from insulin-gene mutations in man (1). By the techniques of protein chemistry and recombinant DNA technology, the abnormal insulins from two of these individuals have been identified as human insulin B25 (Phe → Leu) (human [Leu<sup>B25</sup>]-insulin, insulin Chicago) (1–5) and human insulin B24 (Phe → Ser) (human [Ser<sup>B24</sup>]-insulin, insulin Los Angeles) (1, 6, 7); the structure of the abnormal insulin from the third remains to be determined. Because small amounts of normal insulin (in addition to large amounts of abnormal insulin) were detected in the serum of all three individuals (1), and because restriction endonuclease mapping has shown that two have both normal and abnormal insulin gene alleles (1, 4–6), it seems that normal and abnormal insulin gene alleles are coexpressed in affected subjects. Clinical characteristics of these patients with mutant insulins have been summarized (8) and include hyperinsulinemia; a lack of evidence for insulin resistance due to contra-insulin hormones, insulin antibodies, insulin receptor antibodies, or decreased numbers of insulin receptors; a normal response to exogenous insulin; a reduced biological activity of endogenous insulin; and a reduced C-peptide/insulin molar ratio. Glucose intolerance and overt diabetes may or may not be present.

Although the amino acid substitutions that occur in abnormal human insulins are themselves genetically and chemically important (1, 5–7, 9, 10), the greatest impact of these mutant hormones in studies of normal and abnormal human physiology derives from their altered potential for recognition by cellular insulin receptors and from their reduced ability to stimulate appropriate cellular responses: Human [Leu<sup>B25</sup>]-insulin and human [Ser<sup>B24</sup>]-insulin have only ~2 and 16%, respectively, of the biological potency of normal human insulin (7, 10–12). Determining the consequences of the secretion of low-activity insulins in man thus requires separate consideration of insulin

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secretion rates in response to appropriate stimuli, receptor binding and biological activities of the secreted insulin, and metabolic clearance rates of each circulating hormone form.

The current investigation was prompted by the opportunity to study seven members of a family, the proband of which had been shown to secrete human [Ser<sup>B24</sup>]-insulin (7), and to examine the disposition of semisynthetic human [Ser<sup>B24</sup>]-insulin by using an animal model. Our results show that (a) six of seven family members tested express the abnormal gene for human [Ser<sup>B24</sup>]-insulin, but only two are diabetic; (b) diabetes within this group appears to be associated with both altered rates of insulin secretion and expression of the gene coding for [Ser<sup>B24</sup>]-insulin; and (c) the hyperinsulinemia and the low C-peptide/insulin molar ratio that are characteristic of these patients probably arise from a slow clearance of the abnormal insulin from the circulation.

## Methods

**Subjects and clinical evaluation.** All subjects in this report were between 90% and 110% of ideal body weight and were members of a single family spanning three generations. Approval for these studies was obtained from the Clinical Investigation Committees of the University of Chicago and the University of Southern California. The proband, a 29-yr-old white female, was identified as being glucose intolerant ~3 yr ago by Dr. Wishner (Los Angeles, CA). The proband's 59-yr-old father is an insulin-treated diabetic; her mother (59 yr old), two brothers (26 and 32 yr old), a sister (23 yr old), and a niece (3 yr old) are all asymptomatic. Fasting blood was obtained from each of these subjects for determination of serum immunoreactive insulin and glucose; additional samples of serum were taken for high performance liquid chromatography (HPLC)<sup>1</sup> analysis of serum insulin (see below). Oral glucose tolerance tests (100 g of glucose ingested after an overnight fast) were performed on the proband, a brother, and a sister. Venous blood was drawn for the determination of serum insulin, plasma glucose, and plasma C-peptide before glucose ingestion and at 30, 60, 90, and 120 min after glucose ingestion. Enough blood was obtained from the brother and sister for purification and HPLC analysis of serum insulin at the time of each oral glucose tolerance test.

**Serum insulin purification and HPLC analysis.** Insulin from 2–10 ml of human or dog serum was purified by immunoaffinity chromatography with guinea pig antiporcine insulin antiserum covalently coupled to cyanogen bromide-activated Sepharose 4B (Pharmacia Fine Chemicals Inc., Piscataway, NJ) (1, 13). Insulin was eluted from the column with a 90–95% yield by means of 3 M acetic acid containing 5 µg/ml of bovine serum albumin. Insulin-containing samples were dried under vacuum and the residue was dissolved in 0.1 ml of 3 M acetic acid for analysis by HPLC. The liquid chromatograph (Series 4) and automatic sample injector (ISS-100; both, Perkin-Elmer Corp., Chromatography Division, Norwalk, CT) and the 5-µm ultrasphere ion-pairing column (C-18; Altex Scientific, Inc., Berkeley, CA) have been described before (1). Insulin was eluted from the reverse-phase column at 22°C under isocratic conditions with a flow rate of 1 ml/min and a solution of 29.7% (vol/vol) acetonitrile prepared in an aqueous buffer that contained

0.1 M phosphoric acid, 0.02 M triethylamine, and 0.05 M NaClO<sub>4</sub>, all adjusted to pH 3.0 with NaOH (1). 0.4-ml fractions were collected, dried under vacuum, and reconstituted in borate buffer (14) for radioimmunoassay of insulin.

**Insulin preparations and radioimmunoassays.** Animal infusion studies used biosynthetic human insulin (Humulin; Eli Lilly and Co., Indianapolis, IN) and semisynthetic human [Ser<sup>B24</sup>]-insulin (prepared from porcine deso-octapeptide insulin and a chemically synthesized carboxyl-terminal B chain octapeptide with trypsin-catalyzed peptide-bond formation between Arg<sup>B22</sup> and Gly<sup>B23</sup>) (7). The concentrations of solutions of normal human insulin, human [Ser<sup>B24</sup>]-insulin, and human [Ser<sup>B25</sup>]-insulin (7), used as radioimmunometric standards, were determined by both amino acid analysis and optical absorbance at 275 nm. Methods for the radioimmunoassay of serum insulin (14) and of plasma C-peptide (15) have been described.

**Animal experiments.** Two different conscious-dog models were used in these studies. The first allowed the graded intraportal infusion of mixtures of normal human insulin and [Ser<sup>B24</sup>]-insulin while blood samples were taken from the femoral artery. At laparotomy, infusion catheters were placed in the splenic vein of normal mongrel dogs (17 kg) so that the tips lay just near the portal vein formation. A sampling catheter was placed in the femoral artery and a complete pancreatectomy was performed to eliminate endogenous insulin secretion. The ends of the catheters were placed subcutaneously for easy access. Animals were maintained by one or two injections of neutral protamine zinc Hagedorn insulin per day and by pancreatic enzyme replacement (four tablets of Cotazyme [Organon Pharmaceuticals, West Orange, NJ] per feed). Experiments were performed 3 wk later, when recovery from the surgical procedures was complete. After the dogs had fasted overnight, equimolar amounts of normal insulin and [Ser<sup>B24</sup>]-analogue were infused via the portal vein catheter. The insulin mixture was infused initially at a rate (3.5 pmol/kg per min) designed to approximate the steady state level of immunoreactive insulin found in patients who secrete [Ser<sup>B24</sup>]-insulin; 1 pmol of insulin is equivalent to 145 µU. After 60 min, the dogs were given 25 g of glucose orally and the infusion rate was progressively increased at 5-min intervals, so that by 60 min the rate was 21 pmol/kg per min. The infusion rate was then progressively reduced over an additional 120-min (see Fig. 5) and the basal rate of infusion was resumed for 60 min. Infusion rates were chosen to simulate the rise and fall in peripheral insulin levels that occur in response to the administration of oral glucose in man. Blood samples for the determination of immunoreactive insulin and glucose, and for HPLC analysis of serum insulin, were drawn from a catheter placed in the femoral artery. Arterial blood glucose levels were 98, 286, 126, 89, and 81 mg/dl at 0, 60, 120, 180, and 240 min, respectively, during the period of the graded infusion.

The second conscious-dog model allowed the peripheral infusion of normal human insulin or human [Ser<sup>B24</sup>]-insulin while blood samples were taken from portal vein, hepatic vein, and femoral artery catheters that had been placed 3 wk before each experiment. The experiments were performed in fasted animals during continuous high dosage somatostatin infusion (800 ng/kg per min) to suppress endogenous insulin secretion. After an appropriate bolus injection of the corresponding insulin, normal human insulin or human [Ser<sup>B24</sup>]-insulin was infused via a peripheral vein at a rate of 1.0 or 0.25 pmol/kg per min, respectively, to achieve similar peripheral levels of immunoreactive insulin. Samples of blood were drawn from the femoral artery, portal vein, and hepatic vein at 5-min intervals, beginning 30 min after the start of the insulin infusion to ensure that steady state concentrations had been reached. The infusion was stopped after a total of 50 min had elapsed; samples of blood were drawn at 1- to 5-min intervals from the femoral artery

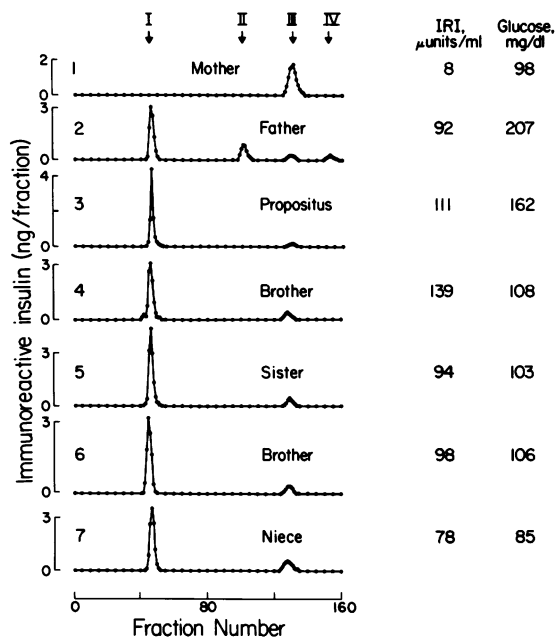
1. Abbreviation used in this paper: HPLC, high performance liquid chromatography.

for an additional 20–30 min to evaluate the half-life of the infused insulin. After levels of infused insulin had been allowed to decay, the same animal was infused with the second of the two insulins using the identical protocol. Hepatic plasma flow, hepatic insulin extraction, and metabolic clearance rate were calculated as previously described (16, 17). Data are expressed as mean  $\pm$  SEM. The order in which the insulins were infused did not affect any of these parameters.

## Results

### HPLC analysis and immunometric detection of serum insulin.

As we have already shown (1, 6, 7), one of the three subjects identified as secreting an abnormal human insulin expresses the mutant insulin gene coding for human [Ser<sup>B24</sup>]-insulin. Initial experiments in the current study were designed to investigate the penetrance of expression of this mutant insulin gene in the family of the propositus. Fig. 1 shows HPLC profiles for immunoaffinity chromatography-purified serum insulin derived from seven family members. The major fraction of serum insulin in six of these individuals elutes from the reverse-phase HPLC column at the position of human [Ser<sup>B24</sup>]-insulin rather than at that of normal human insulin. The mother of the propositus

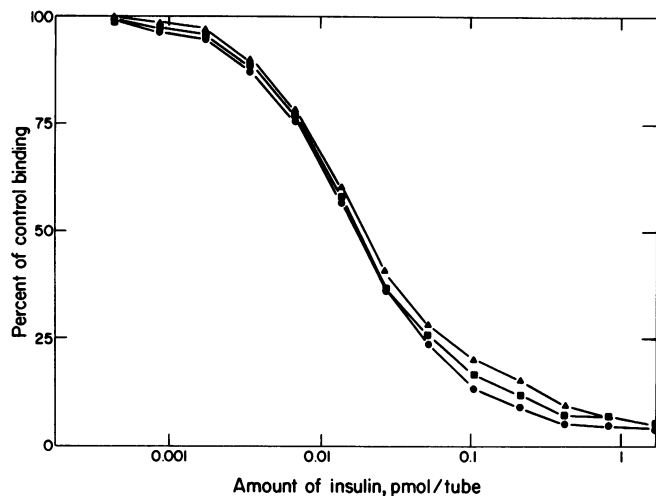


**Figure 1.** HPLC analysis of serum insulin from the propositus and six members of her family. Serum insulin was purified by immunoaffinity chromatography and was subjected to HPLC and further analysis as described in Methods. Amount of immunoreactive insulin is plotted against HPLC fraction number in each of the profiles. Arabic numerals identify the individual with respect to subsequent experiments; subject 3 is the propositus. Roman numerals I–IV identify the elution positions of human [Ser<sup>B24</sup>]-insulin, bovine insulin, normal human insulin, and porcine insulin, respectively. Columns to the right of the figure list the fasting serum levels of immunoreactive insulin and glucose determined for each of the seven subjects.

(subject 1), however, secretes only the normal hormone. In each of subjects 2 through 7, the ratio of the serum concentration of abnormal insulin to that of normal insulin is  $>9:1$ . Note that the immunoaffinity chromatography-purified serum insulin from the father of the propositus (subject 2), an insulin-treated diabetic patient, consists of large amounts of human [Ser<sup>B24</sup>]-insulin, small amounts of normal human insulin, and small amounts of beef insulin and pork insulin, the last two of which result from exogenous insulin therapy. As noted before (8), all subjects who secrete the abnormal human insulin show marked fasting hyperinsulinemia, whereas only two of the subjects, the propositus and her father (subjects 3 and 2), show fasting hyperglycemia.

Taken together, the results of Fig. 1 show that the expression of the mutant gene that codes for human [Ser<sup>B24</sup>]-insulin, like the existence of the abnormal gene itself (6, 8), spans three generations within a single family; that the abnormal insulin gene is inherited in an autosomal dominant pattern; and that the association of the expression of this gene with diabetes is incomplete. Quantitative determination of serum insulin concentrations in subjects who secrete [Ser<sup>B24</sup>]-insulin depends, however, on the immunometric equivalency of normal human insulin and the Ser<sup>B24</sup>-substituted analogue. As shown in Fig. 2, normal human insulin, human [Ser<sup>B24</sup>]-insulin, and human [Ser<sup>B25</sup>]-insulin show little difference in their relative binding affinities for the antiinsulin serum, which is now in use. Thus, our radioimmunoassay can be used to quantitate human [Ser<sup>B24</sup>]-insulin and normal human insulin without need for correction, at least for amounts of insulin  $< \sim 0.05$  pmol/assay tube. Note, however, that other insulin radioimmunoassays appear to be more sensitive in discriminating insulin analogues substituted at position B24 or B25 (18) and that immunometric equivalency must be separately assessed for each new or uncommon insulin analogue under investigation.

**Responses to oral glucose.** To examine in detail the glucose responses of both diabetic and nondiabetic members of the family that secretes human [Ser<sup>B24</sup>]-insulin, subjects 3, 4, and 5 (Fig. 1) were given oral glucose tolerance tests. Results of these tests, which documented circulating levels of insulin, C-peptide, and glucose, are presented in Fig. 3. It is important that all three subjects showed fasting hyperinsulinemia. The propositus (subject 3) also showed fasting hyperglycemia and the marked elevations in plasma glucose levels typical of a diabetic patient after glucose ingestion. In contrast, her two siblings (subjects 4 and 5) exhibited a glucose tolerance that approached that expected for normal individuals: Despite an apparently delayed response (to 90 min) in attaining peak insulin levels, and despite the higher-than-normal serum concentrations of insulin (2 pmol/ml; normal = 0.3–0.8 pmol/ml) and C-peptide (4 pmol/ml; normal = 0.7–2.0 pmol/ml) eventually reached (19–21), most of the glucose levels in subjects 4 and 5 were within the normal range throughout the 2-h period. In this single comparison, increments in insulin and C-peptide, summed from measurements 30, 60, and 120 min after glucose ingestion



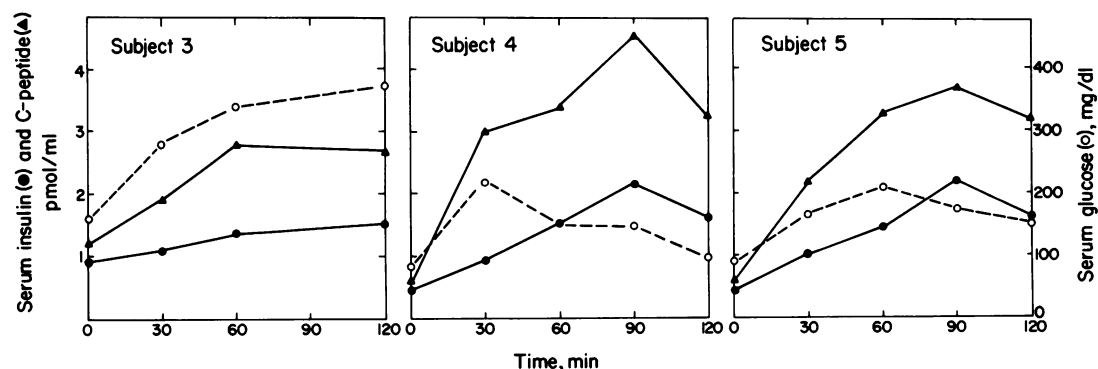
**Figure 2.** Radioimmunoassay standard curves for normal human insulin and for insulin analogues bearing serine-for-phenylalanine substitutions. Assay conditions were those used for the immunoassay of serum insulin as described in Methods. Data are shown for biosynthetic normal human insulin (●), semisynthetic human [Ser<sup>B24</sup>]-insulin (■), and semisynthetic human [Ser<sup>B23</sup>]-insulin (▲). Control binding is defined as the amount of [<sup>125</sup>I]-iodoinsulin bound to antibody in the absence of competitor. Amounts of competitor present in each radioimmunoassay tube are plotted on the abscissa.

were, respectively, 1.3 and 3.8 pmol/ml for subject 3; 2.8 and 7.8 pmol/ml for subject 4; and 2.8 and 6.9 pmol/ml for subject 5. Thus, it appears that the B cell secretion response of the diabetic propositus was only about half that of her two nondiabetic siblings over the period of the test. All three subjects showed C-peptide/total insulin molar ratios of ~1.5 in the fasting state (normal = 4–16, reference 20) and all showed transient elevations in that ratio (maximum values, 2.0–3.3) during the course of the glucose tolerance test; normal individuals show

transient depressions in the ratio after taking oral glucose (19, 21).

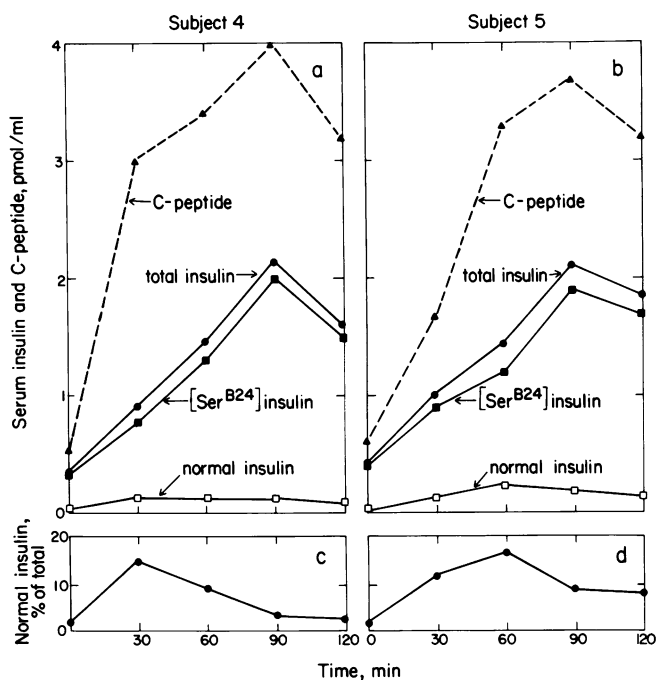
Enough serum remained from the glucose tolerance tests performed on subjects 4 and 5 to assess separately the secretion of human [Ser<sup>B24</sup>]-insulin and normal human insulin in response to oral glucose. Serum insulin from each time point was purified by immunoaffinity chromatography and subjected to HPLC analysis, which yielded profiles similar to those shown in Fig. 1. The results of this analysis are presented in Fig. 4, using the common format for presenting glucose tolerance data. As shown in *a* and *b*, the abnormal form of the hormone, human [Ser<sup>B24</sup>]-insulin, accounts for the major fraction of serum insulin at each time point. Further, for each of these two subjects, the fraction of serum insulin attributable to normal human insulin rose from the basal state upon ingestion of oral glucose and fell again while the level of total insulin continued to increase. In fact, the levels of normal insulin during fasting (0.04 pmol/ml, ~6 microunits/ml), the rise in levels of normal insulin during early periods (to peak values of 0.23 pmol/ml, ~33 microunits/ml), and the fall in levels of normal insulin to near basal values during later periods are indicative of a relatively normal response to oral glucose; the character of the curves that reflect human [Ser<sup>B24</sup>]-insulin secretion in response to oral glucose, and the high circulating levels of [Ser<sup>B24</sup>]-insulin eventually reached are, however, clearly abnormal. Although the C-peptide/abnormal insulin molar ratio rose in parallel with the C-peptide/total insulin molar ratio during glucose stimulation of B cell secretion, the C-peptide/normal insulin molar ratio fell to 15–30% of its basal value under the same circumstances.

**Animal models.** The data in Fig. 4 show that the hyperinsulinemia present in subjects 4 and 5, both during fasting and after stimulation with oral glucose, is due to the abnormal hormone human [Ser<sup>B24</sup>]-insulin, rather than to normal human insulin. Although this hyperinsulinemia and the related high [Ser<sup>B24</sup>]-insulin/normal insulin molar ratio found in the circulation could have arisen from nonequivalent storage and se-



**Figure 3.** Insulin, C-peptide, and glucose responses of three subjects during an oral glucose tolerance test. Subject 3 is the diabetic propositus, subjects 4 and 5 are two of her nondiabetic siblings. Details of the measurements and of the oral glucose tolerance test are provided

in Methods. Insulin (●) and C-peptide (▲) concentrations are plotted using the left-hand ordinate scale, glucose concentrations (○) using the right-hand ordinate scale; the abscissa shows the time after the administration of oral glucose that blood was drawn for analysis.

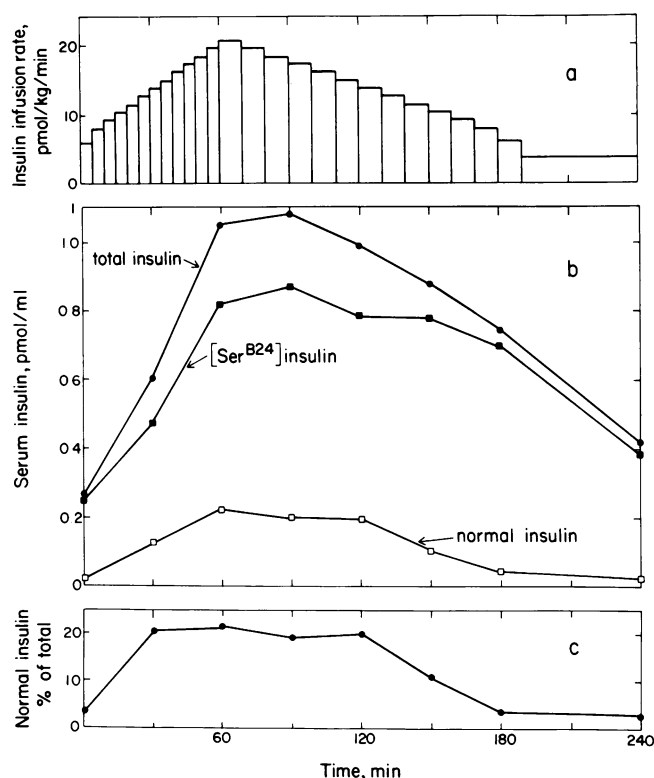


**Figure 4.** HPLC analysis of serum insulin derived from an oral glucose tolerance test. Data from two subjects (subjects 4 and 5) are shown; curves for C-peptide and total insulin in *a* and *b* are the same as those presented in Fig. 3. Serum samples from each time point were subjected to immunoaffinity chromatography for purification of insulin; purified insulin was analyzed by HPLC as described in Methods to separate and quantitate human [Ser<sup>B24</sup>]-insulin (the abnormal insulin) and normal human insulin (*a* and *b*). *c* and *d* show the percentage of total insulin identifiable as normal human insulin at each time point for subjects 4 and 5, respectively. The abscissa records the time after administration of oral glucose in each case.

cretion of the two insulin forms, our data from an earlier patient suggested that normal and abnormal insulins were stored and secreted in approximately equimolar amounts (1–3). The availability of semisynthetic human [Ser<sup>B24</sup>]-insulin and biosynthetic normal human insulin permitted us to test, in an animal model, whether cosecretion of the normal and abnormal insulins in equimolar amounts could account for the hyperinsulinemia of subjects who express the gene for human [Ser<sup>B24</sup>]-insulin. The model involves the graded intraportal infusion of an equimolar mixture of human [Ser<sup>B24</sup>]-insulin and normal human insulin in a pancreatectomized dog, by using a protocol designed to simulate insulin delivery from the pancreas under both basal and stimulated conditions.

A steady state infusion of the equimolar mixture of normal and abnormal insulins at a combined rate of 3.3 pmol/kg per min over a 60-min period resulted in a basal total serum insulin level of 0.37 pmol/ml (53 microunits/ml). Increasing and then decreasing the rate of infusion over a 4-h period (to mimic pancreatic insulin secretion associated with the administration of oral glucose; Fig. 5 *a*) resulted in total serum insulin levels

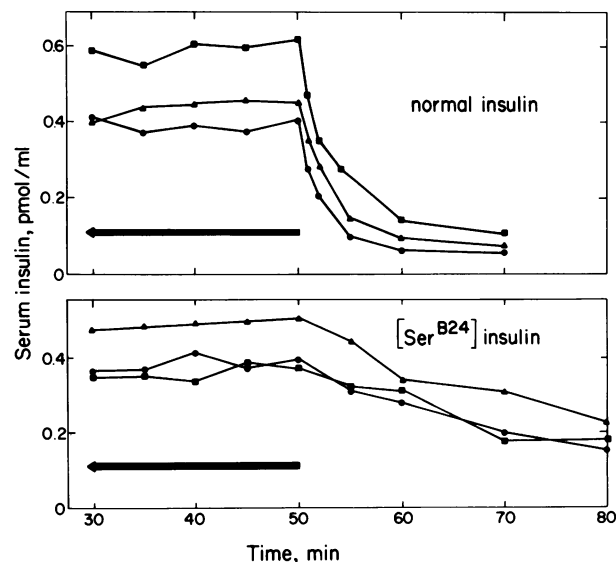
that increased approximately fourfold by 60 min and then returned to near basal values by the end of the experiment (Fig. 5 *b*). Although the normal and abnormal insulins were infused at equimolar concentrations, HPLC analysis of serum insulin at each of the time points shown in Fig. 5 demonstrated that the abnormal insulin always predominated in the peripheral circulation (Fig. 5 *b*). Less than 5% of total serum insulin in the basal state was attributable to the normal hormone; even at its peak level, normal insulin accounted for  $\leq 20\%$  of total circulating insulin. Fig. 5 *c* shows that the fraction of total insulin attributable to the normal hormone rose rapidly early in the experiment, reached a transient steady state, and then fell while total insulin levels were still elevated. The high levels of total



**Figure 5.** HPLC analysis of serum insulin derived from the graded intraportal infusion of an equimolar mixture of human [Ser<sup>B24</sup>]-insulin and normal human insulin in a pancreatectomized dog. Details of the animal preparation are described in Methods. *a* shows the rate of infusion of total insulin for each time segment after a 60-min period in which the rate of infusion was 3.3 pmol/kg per min. *b* shows the results of HPLC analysis applied to individual serum samples to separate and quantitate human [Ser<sup>B24</sup>]-insulin and normal human insulin. *c* shows the percentage of total insulin identifiable as normal human insulin at each time point. Data at time zero show values at the close of the constant basal infusion. The abscissa records the time after initiation of the graded intraportal infusion. Similar results were found for two other dogs infused with the insulin mixture in an equivalent way.

insulin achieved, the low fraction of total insulin attributable to normal insulin, and the rapid rates of change of normal insulin levels in this experiment all mimic results obtained in man (see Fig. 4).

Because the rates of infusion of human [Ser<sup>B24</sup>]-insulin and normal human insulin were equal in the experiment shown in Fig. 5, the elevated levels of total serum insulin and the low fraction of total insulin attributable to normal insulin most likely arose from different rates of clearance of the two insulin forms. Fig. 6 compares the steady state plasma concentrations and half-lives of normal human insulin and human [Ser<sup>B24</sup>]-insulin in somatostatin-treated dogs. Nearly equivalent steady state levels of circulating hormone were achieved by the peripheral infusion of normal insulin at a rate of 1.0 pmol/kg per min and by the peripheral infusion of human [Ser<sup>B24</sup>]-insulin at a rate of 0.25 pmol/kg per min; it seems, therefore, that the overall metabolic clearance rate for the abnormal insulin is only about one-fourth that for the normal hormone. Calculated values for metabolic clearance rates ( $20.8 \pm 2.3$  and  $6.0 \pm 0.6$  ml/kg per min for normal insulin and [Ser<sup>B24</sup>]-insulin, respectively) and for hepatic extraction ( $43.6 \pm 5.8$  and  $3.2 \pm 2.4\%$  for normal insulin and [Ser<sup>B24</sup>]-insulin, respectively [ $n = 3$  in both cases]) confirm



**Figure 6.** Metabolic clearance and half-life of normal human insulin and human [Ser<sup>B24</sup>]-insulin in dogs. Details of the animal model are described in Methods. Animals received an appropriate bolus injection of either normal insulin or the [Ser<sup>B24</sup>]-analogue, which were administered by constant intravenous infusion at rates of 1.0 and 0.25 pmol/kg per min, respectively. After 30 min of infusion, samples were drawn from the femoral artery at 5-min intervals for determination of serum insulin. After five such samples had been collected, the infusion was terminated and additional samples were drawn from the femoral artery at the intervals shown in the figure. Each of the two peptides was infused into each of three dogs (identified by ●, ■, or ▲) in random order to minimize interanimal variation.

this result. The different decay rates of the levels of the two insulins after steady state infusion also indicate a slower clearance of the abnormal insulin; as illustrated in Fig. 6, the half-life of human [Ser<sup>B24</sup>]-insulin under these circumstances ( $\sim 17$  min) is almost six times greater than the half-life of normal human insulin ( $\sim 3$  min). Taken together, these results demonstrate the markedly slowed clearance of the abnormal hormone analogue relative to that of the normal hormone during its circulation in vivo.

## Discussion

The present results on the expression of the mutant insulin gene coding for human insulin B24 (Phe  $\rightarrow$  Ser) (insulin Los Angeles) extend previous reports on the identification of the abnormal insulin (1, 7), the determination of the structure of the abnormal insulin gene (6), and the occurrence of the mutant allele in five members of the affected family (8). Thus, six of seven members of the family, a group spanning three generations, have now been shown to secrete the abnormal insulin; in each case  $\geq 90\%$  of total serum insulin during fasting arises from the expression of the mutant allele and the secretion of human [Ser<sup>B24</sup>]-insulin, whereas  $\leq 10\%$  arises from the expression of the normal allele and the secretion of normal human insulin. Note that the abnormal gene arises from one insulin gene allele of the father of the propositus; the mother of the propositus maintains two normal insulin gene alleles and secretes only normal insulin; and all four progeny have apparently inherited the abnormal insulin gene from their father and a normal insulin gene from their mother (reference 8 and this report). As shown in Figs. 1 and 3, however, only two of the six affected members of the family are diabetic. The glucose tolerance curves of Fig. 3 suggest that diabetes in the case of the propositus arises from a low responsiveness of the B cell to glucose stimulation, in addition to the secretion of human [Ser<sup>B24</sup>]-insulin. Note that two siblings who secrete the same abnormal insulin/normal insulin mixture in amounts greater than those secreted by the propositus show no major degree of glucose intolerance. It is important to study further the extent to which each of these insulins contributes to glycemic control.

Although an individual who secretes equivalent amounts of normal insulin and low activity abnormal insulin probably would be under an unusual burden in terms of secretion of the hormone in physiologically effective amounts, the cause of diabetes in the propositus is not clear. It is important to learn whether this subject's decreased B cell response (relative to that of her siblings) is secondary to hyperglycemia (and might be correctable, at least in part, by insulin therapy) and whether nondiabetic carriers of the mutant insulin gene coding for [Ser<sup>B24</sup>]-insulin are more likely to develop glucose intolerance in the future.

The association of hyperinsulinemia with the secretion of human insulin B24 (Phe  $\rightarrow$  Ser) could arise from either a decreased rate of hepatic insulin extraction, as has been suggested for the hyperinsulinemia found under certain conditions of obe-

sity (21) and mild glucose intolerance (22), or an increased rate of insulin secretion. Three findings reported in Figs. 3 and 4 must be considered in evaluating these possibilities: First, the major fraction of serum insulin in subjects 4 and 5 is attributable to human [Ser<sup>B24</sup>]-insulin under conditions of both fasting euglycemia and the transient hyperglycemia that results from glucose ingestion; second, the low fraction of serum insulin attributable to normal human insulin in the basal state is seen to rise sharply during glucose stimulation and then to decrease to near fasting levels while total insulin levels are still increasing; third, the diabetic propositus maintains as high a fasting level of total insulin as her nondiabetic siblings, although her response to oral glucose seems diminished. It is important that the findings noted above (including the high steady state levels of total serum insulin reached at low insulin delivery rates) are easily simulated by the graded intraportal infusion of an equimolar mixture of human [Ser<sup>B24</sup>]-insulin and normal human insulin in dogs. Together with findings in the animal model that demonstrate the decreased hepatic extraction and slowed metabolic clearance of human [Ser<sup>B24</sup>]-insulin relative to that of the normal hormone, these data lead us to suggest that the hyperinsulinemia of subjects who secrete the abnormal hormone is due to the decreased hepatic extraction of human [Ser<sup>B24</sup>]-insulin. The relatively normal fasting C-peptide levels observed in affected individuals are consistent with hyperinsulinemia's being due to decreased hepatic extraction of the abnormal insulin, rather than to increased rates of secretion of either human [Ser<sup>B24</sup>]-insulin or normal human insulin.

A number of studies have demonstrated (a) the need for interaction of insulin with its cellular plasma membrane receptor for subsequent hormone metabolism by the liver (23, 24), (b) the saturation of hepatic insulin metabolism by increasing concentrations of the hormone (25), (c) the regulation of hepatic insulin extraction by physiological stimuli (16), and (d) the structural specificity of receptor-linked mechanisms for hepatic insulin metabolism (10). Interaction of insulin with its receptor therefore seems to be a prerequisite for its metabolism and degradation, as well as for its action. Since human [Ser<sup>B24</sup>]-insulin has only 16% of the biological activity of normal insulin (7), it is not surprising that its rate of metabolic clearance through the liver would be equivalently depressed. On the other hand, the C-peptide (which for subjects of this study is secreted in molar amounts equal to the sum of human [Ser<sup>B24</sup>]-insulin and normal human insulin) is not subject to hepatic metabolism (26). Given relatively normal fasting C-peptide levels in patients who secrete abnormal insulins, the low fasting C-peptide/insulin molar ratio typical of such individuals undoubtedly arises mostly from the slow metabolic clearance and high serum levels of the abnormal hormone. Similarly, the delay with which peripheral levels of [Ser<sup>B24</sup>]-insulin reach a maximal value during B cell stimulation (relative to the period required for normal insulin; Fig. 4) results from the differential rates of clearance of human [Ser<sup>B24</sup>]-insulin and normal human insulin.

These studies represent only a first step in investigating the

physiological consequences of the secretion of low activity abnormal insulins in man. The special opportunities to examine a large group of affected subjects, serum insulin (by HPLC methods that separate normal and abnormal forms), the metabolic fate of infused semisynthetic human [Ser<sup>B24</sup>]-insulin, and animal models that mimic both basal and stimulated insulin secretion have been instrumental in this regard. From a variety of experiments, it appears that the prolonged half-life of human [Ser<sup>B24</sup>]-insulin can alone account for many of the clinical findings associated with both the diabetic propositus and her nondiabetic siblings. The relationship between abnormal insulin secretion and diabetes, and the biological activities of the abnormal insulins in vivo are important issues that remain to be studied. In fact, a complete understanding of abnormal insulin physiology in man will have to deal separately with the questions of biosynthesis, secretion, action, and clearance of three distinct B cell products: normal insulin, abnormal insulin, and C-peptide.

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