Immunogenicity of Insulin

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SUMMARY

Highly purified bovine and porcine insulins (single component insulin) did not produce antibodies in rabbits, and the highly purified porcine insulin was less immunogenic in diabetic patients than USP porcine insulin. In rabbits one porcine high molecular weight fraction (peak A) and two bovine high molecular weight fractions (peak A and peak B) separated from USP insulin by Sephadex chromatography were immunogenic, producing increases in the amount of ^{125}I-labeled single component insulin bound by the serum. Both peaks (A and B) derived from bovine insulin produced significantly greater insulin binding in rabbits than did the same peaks derived from porcine insulin. In diabetic patients single component porcine insulin produced less binding of insulin to the serum than did USP insulin. DIABETES *21* (Suppl. 2):657-60, 1972.

Berson and Yalow[1,2] first demonstrated that antibodies to insulin were present in the sera of insulin-treated diabetic patients. In 1962 Prout[3] reviewed the literature concerning insulin antigenicity. Until recently the development of antibodies to insulin in insulin-treated diabetic patients has been considered inevitable and the consequence of repeated injections of a non-homologous polypeptide. However, recent studies by Schlichtkrull et al.[4] and by us suggest that certain higher molecular weight components in commercial insulin preparations may be largely responsible for this antibody formation and that the immunogenicity of highly purified insulin preparations is of a low order of magnitude.

Chromatography of USP porcine insulin by G-50 (Fine) Sephadex in molar acetic acid yields two UV 276 mμ. peaks containing materials of greater molecular size than insulin (figure 1). Polyacrylamide disc-gel electrophoresis of the fractions reveals the complex heterogeneity of these materials. Peak A consists of proteins with molecular weights greater than 10,000. Some of these components cross-react in both the insulin and proinsulin radioimmunoassays. Peak B contains proinsulin and structurally related materials plus a "dimer" of insulin. Peak D consists mainly of "pure" insulin plus the desamido insulins and the arginine insulins;[5] we refer to this chromatographic fraction as single-peak insulin (SPI).

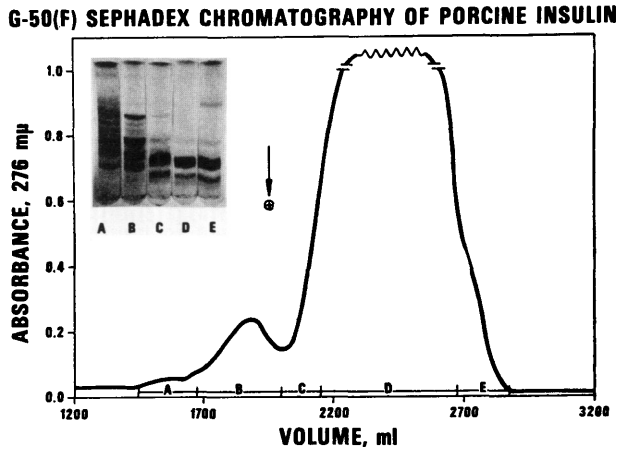

FIG. 1. G-50 Sephadex (Fine) chromatographic elution profile obtained with a gram of crystalline porcine insulin on a 5 X 185 cm. column in molar acetic acid at 4° C. The fractions were pooled as shown and lyophilized. The relative yields were as follows: Peak A, 1.6 per cent; peak B, 6.9 per cent; peak C, 5.4 per cent; peak D, 80.7 per cent; peak E, 5.4 per cent. Polyacrylamide disc-gel electrophoresis profiles for these peak preparations (see inset photograph) were determined with 20 per cent gels as described previously[8] using 70 μg. sample loads.

The immunogenicity of insulin was studied in rabbits and in diabetic patients with methods developed by Schlichtkrull and associates.[6,7] In the radioimmunoassay for measurement of antibody concentration, each assay tube contained 0.1 ml. of undiluted serum and 0.2 ng. ^{125}I-labeled single component insulin. The antibody titer was recorded as the percentage of ^{125}I-labeled insulin bound. The sensitivity of this assay is such that even 50

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per cent binding represents an insignificant antibody titer in terms of clinically demonstrable insulin resistance. When antibody titer was measured in serum samples from patients, a pool of normal rabbit serum was used as a zero (no binding) control. The use of this control serum usually produced a value above or below zero for the initial (pretreatment) samples of serum from each patient. The following additional controls were included in each assay to monitor changes in sensitivity: several dilutions of a pool of high-titer guinea pig anti-insulin serum and a pool of low-titer rabbit anti-insulin serum.

The six different porcine preparations administered to normal rabbits are listed in table 1. Single component insulin (SCI) is prepared by a combination of DEAE-cellulose and G-50 (Fine) Sephadex chromatography[8] and is comparable in purity to the monocomponent insulin discussed by Schlichtkrull et al.[4,7]

Each of the six porcine preparations was injected subcutaneously at a dose of 40 μg. per animal into three rabbits three times a week. Each rabbit was bled once a week and the serum was separated and stored at —20° C. until assayed for antibody titer. The results, presented in figure 2, show that only peak A material produced significant antibody titer.

A similar experiment in rabbits with materials prepared from bovine insulin is shown in figure 3. In this experiment the A peak was divided into two components,

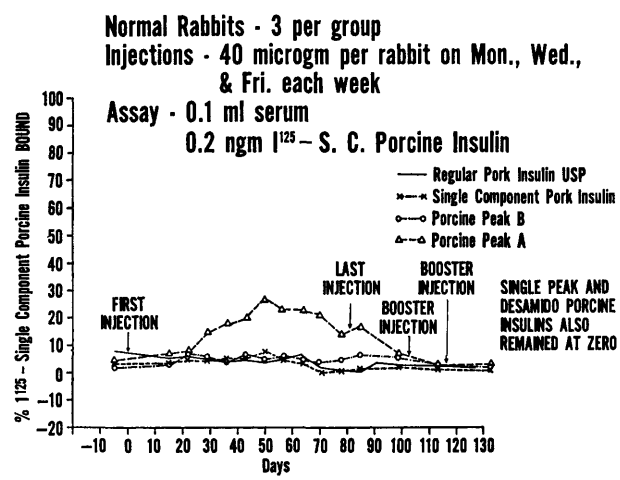

FIG. 2. Immunogenicity of porcine insulin preparations in normal rabbits. Each point is a mean value for three rabbits.

A_1 and A_2, and the desamido insulin was omitted from the treatments. Both A components and peak B material from bovine insulin produced significant binding of labeled single component bovine insulin.

A comparison of the curves in figures 2 and 3 suggests that, in rabbits, bovine A and B peaks are more immunogenic than porcine A and B peaks.

The studies in diabetic patients were made with only

TABLE 1

Preparations used for study of immunogenicity in rabbits

Peak A	High molecular weight material separated from USP insulin on Sephadex G-50(F). See figure 1, fraction A.	
Peak B	Proinsulin and proinsulin-like components separated from USP insulin on Sephadex G-50(F). See figure 1, fraction B.	
USP insulin (23.3 U./mg.)	Contains: Insulin-like*	~92%
	Proinsulin-like	~ 6%
	Insulin aggregates	~ 1%
	Non-insulin	~ 1%
Single peak insulin (SPI) (26.3 U./mg.)	Contains: Insulin-like*	~99%
	Non-insulin	< 1%
Single component insulin (SCI) (28.3 U./mg.)	Contains: "Pure" insulin	>99%
Monodesamido insulin (26.5 U./mg.)	Aspartic acid in place of asparagine at position A_{21}	

*Comprised of "pure" insulin, desamido insulins, arginine insulins, esterified insulins, etc.

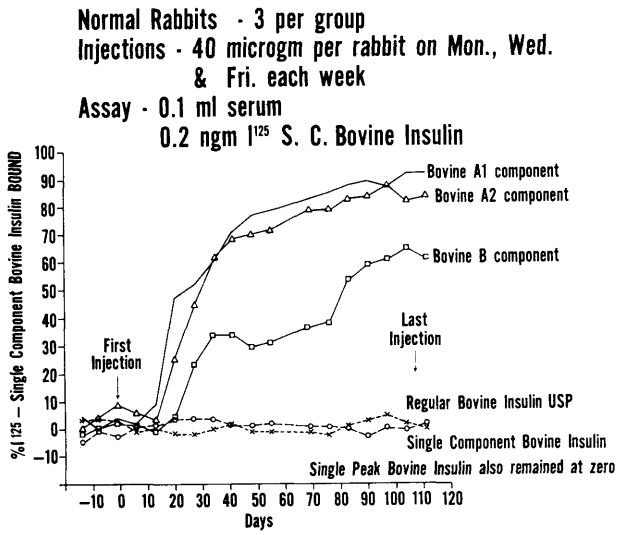

FIG. 3. Immunogenicity of bovine insulin preparations in normal rabbits. Each point is a mean value for three rabbits.

two insulin preparations, USP porcine insulin and single component porcine insulin. The patients were newly diagnosed diabetics who had not previously received any insulin and who were subsequently treated with either USP porcine insulin or single component porcine insulin. The insulin formulations administered were unmodified insulin, Lente® insulin or a combination of the two. Serum samples were collected on each visit to the physician during the first six months of treatment and were kept frozen until assayed for antibody titer. Figures 4 and 5 show the results obtained in eight patients treated by one physician for a six-month period.* Four of the patients received USP porcine insulin (figure 4), and all developed significant insulin binding. The other four patients were treated with single component porcine insulin (figure 5). One of these patients did not develop any insulin binding during six months of treatment. Sera of the other three patients showed some insulin binding; but, during the six-month period of treatment, the degree of binding was very much less than that developed by the patients on USP porcine insulin. The doses of insulin administered to the two groups of patients were similar, although comparisons are difficult, since these patients were children or teenagers and body weight ranged from 17 to 65 kg. When the daily dose was calculated as units per kg., the widest scatter was in the group receiving USP porcine insulin. Patient C.A. was receiving 1.8 U./kg. and R.C., 0.23 U./kg. The range for the group on single component porcine insulin was from 0.31 to 0.40 U./kg. More data from larger groups of patients will be required in order to assess the influence of insulin dose on the development of antibodies to insulin.

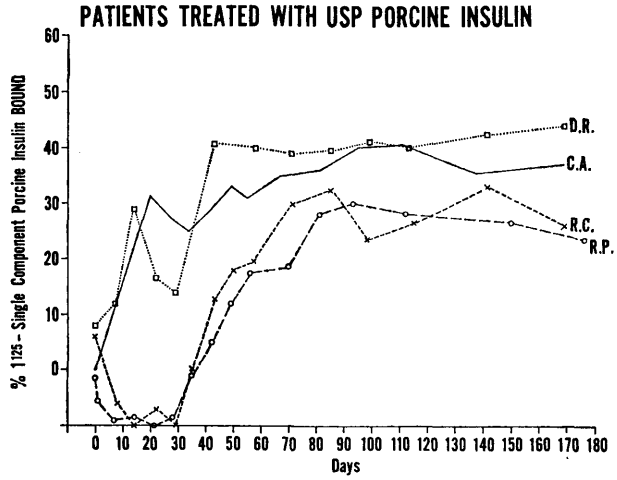

FIG. 4. Immunogenicity of USP porcine insulin in four diabetic patients. See text for details.

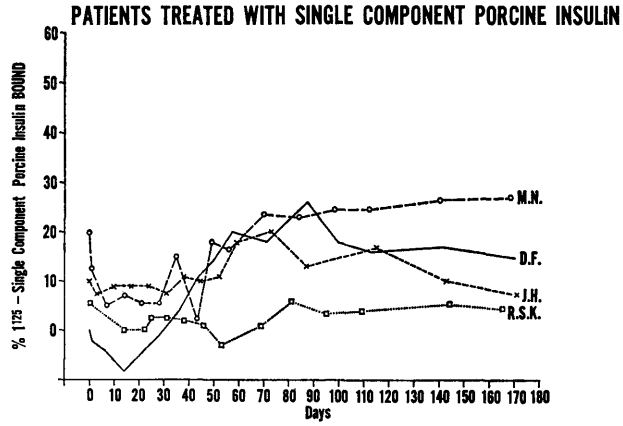

FIG. 5. Immunogenicity of single component porcine insulin in four diabetic patients. See text for details.

From these studies we concluded that, in rabbits, the high molecular weight components contained in USP insulin are more immunogenic than highly purified insulin and that removal of these components makes insulin less immunogenic in patients.

*We are indebted to Dr. Wendell Brown, Indianapolis, for his assistance in the selection of patients and collection of serum samples for this study.

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Summary of Discussion

Dr. Strath Wilson expressed the view that, while the ultrapurification of insulin is no doubt desirable, antigenicity might better be attacked by altering the molecule. He cited, as an example, recent German trials of single component compared with Regular insulin in which there was no difference in the frequency of urticaria. He remarked that the insulin-resistant patients of Dr. Schlichtkrull and Dr. Root showed no difference in insulin requirement whether given Regular, Lente or highly purified insulin. Dr. Wilson would like to see experiments with the production of antibodies to the hormone itself instead of efforts simply to remove the impurities. He recalled that Maloney in Toronto has reduced the antigenicity of the hormone by modifying the molecule with maleic anhydride. Referring to Dr. Davidson's discussion (page 648), Dr. Wilson agreed that sulfated insulin does seem less antigenic than bovine insulin but does not induce tolerance, whereas maleyl insulin does.

Dr. E. F. Pfeiffer reported a patient whose requirement of 800 units of Regular insulin per day dropped to 60 units per day with monocomponent insulin.

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