

INSULIN ALLERGY AND INSULIN RESISTANCE

Report of a Case

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ALLERGIC reactions to commercial insulin preparations were noted soon after the introduction of insulin therapy for diabetes mellitus. While apparently more frequent with the earlier, less-refined insulins, allergic manifestations continue to occur with the relatively pure preparations currently available. The majority of these reactions are limited and local. Certain individuals, however, may manifest generalized, clinically significant, allergic responses.

Insulin resistance* frequently accompanies overt insulin allergy. While insulin resistance may have many causes, it is clear that an immunologic mechanism is operative in certain instances. The present report concerns a patient with generalized insulin allergy who also demonstrated decreasing responsiveness to insulin.

CASE REPORT

The patient was a 39-year-old white woman who was admitted to the hospital in diabetic coma in April, 1958 (Fig. 1). There was a family history of diabetes. The patient had no glycosuria during a pregnancy in 1955. During the 3 months preceding admission, the patient had symptoms of uncontrolled diabetes and sustained a weight loss of forty pounds. Following correction of the ketoacidosis, the patient was discharged in good control on 40 units of NPH insulin daily.

Mild ketoacidosis resulted in hospitalization elsewhere in December, 1958, and January, 1959. Control was obtained with three injections of regular insulin daily. NPH insulin therapy was subsequently reinstituted during hospitalization in April of 1959 for radiation therapy of a grade 2 squamous cell carcinoma of the cervix. Good diabetic control was obtained with 45 units daily. Between May and July of 1959, the patient was seen frequently in the outpatient department because of poor control. In spite of a gradual increase in the daily insulin dose to 80 units of NPH, hyperglycemia, glycosuria, and occasional ketonuria persisted. The patient reported local, transitory urticarial reactions at the site of insulin injections for several months. Areas typical of lipohypertrophy were observed at the sites of repeated insulin injections on both thighs.

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*Arbitrarily defined as a diabetic state lasting longer than 48 hours in which the daily insulin requirement exceeds 200 units and which is not associated with infection or other apparent stress.¹

Mild ketoacidosis developed in July, 1959, and she was again hospitalized. Ten minutes after the intravenous injection of 30 units of regular insulin, the patient developed generalized urticaria. This responded promptly to parenteral antihistamine. Fair diabetic control, without generalized urticaria, was later achieved with subcutaneous injections of insulin. Occasional local urticaria was noted at the site of subcutaneous insulin injection. A trial with tolbutamide was unsuccessful. Phenethylidiguanide was given in addition to insulin and the patient was discharged in fair control on 75 mg. plus 35 units of NPH insulin daily.

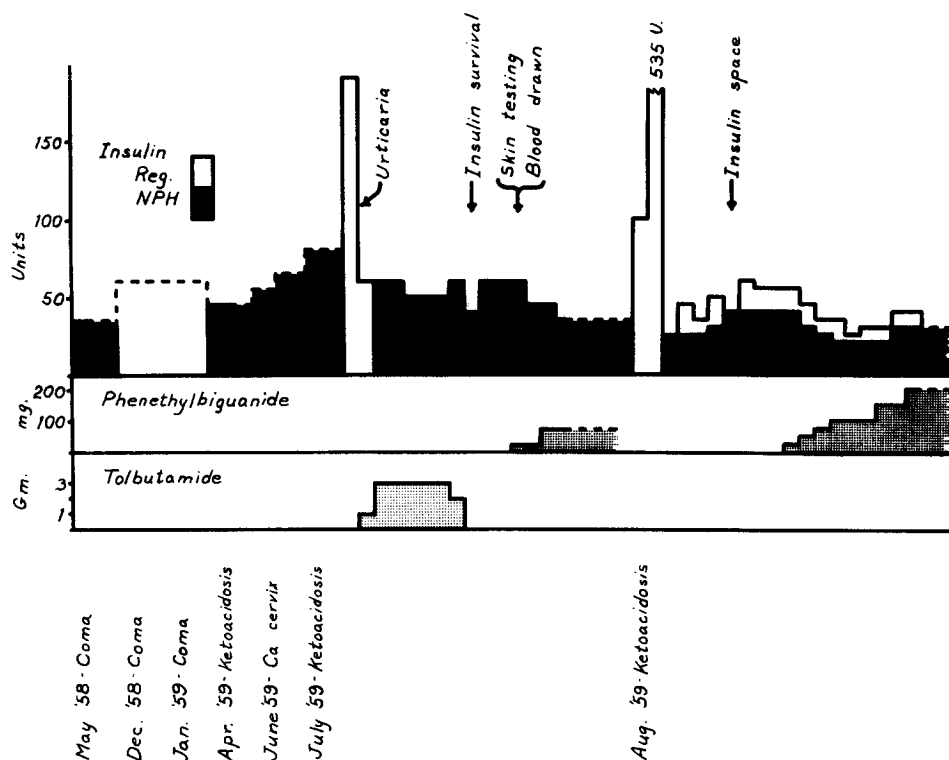


Fig. 1.—Clinical course of patient showing sequence of events and studies in relation to therapy.

Five days later, the patient re-entered the hospital in ketoacidosis. During the first 12 hours of therapy she received 475 units of regular insulin subcutaneously without improvement. Regular insulin was then added to an intravenous infusion and administered slowly. After the infusion of 150 units of insulin over a period of 90 minutes, ketonuria and glycosuria ceased. No allergic manifestations were noted. During the following day, 25 units of NPH insulin was sufficient to prevent glycosuria. On succeeding days, the patient's insulin requirements gradually rose to 55 units daily. She was discharged 2 weeks later on 200 mg. of phenethylidiguanide and 40 units of NPH insulin daily.

There was no history of other allergic manifestations in the patient or in members of her family. Results of skin tests with the usual inhalant antigens were negative.

SPECIAL STUDIES

An attempt was made to characterize the antigen-antibody system responsible for the patient's allergic manifestations and to seek a relationship between this phenomenon and the increasing insulin requirement.

Serum used in the following studies was obtained in the fasting state 24 hours after the last dose of insulin on August 10 (Fig. 1). In vivo procedures were performed in the fasting state 24 hours after the last dose of insulin on August 7 (insulin survival) and August 24 (insulin space).

Skin Sensitizing Antibodies.—Direct intradermal testing with 0.02 ml. of serial dilutions of regular, NPH, PZI, and Lente insulin preparations gave responses which appeared to be roughly proportional to the amount of soluble insulin in each preparation (Table I). Beef, pork, and sheep *insulins** all evoked skin reactions, but extracts of *proteins*† from these species did not elicit responses.

TABLE I. RESULTS OF DIRECT INTRADERMAL TESTING WITH REGULAR AND MODIFIED INSULIN PREPARATIONS DEMONSTRATING SKIN SENSITIZING ANTIBODIES*

CONCENTRATION (UNITS/ML.)	REGULAR	NPH	PZI	LENTE
8	++++	+	+++	+++
0.8	+++	+	+	++
0.08	++	—	+	+

*Reactions were graded 15 minutes after injection of 0.02 ml. of the test material.

Two nonallergic, nondiabetic subjects were then passively sensitized with 0.1 ml. aliquots of the patient's serum. The sensitized sites were challenged 48 hours later with dilutions of the same four insulin preparations (8 units per milliliter). The responses were identical to those observed on direct testing (Table II). There were no reactions to identical dilutions of the insulin preparations at control sites.

TABLE II. RESULTS OF PASSIVE TRANSFER TESTING DEMONSTRATING TRANSFERABLE SKIN SENSITIZING ANTIBODIES*

	REGULAR	NPH	PZI	LENTE
Sensitized site	+++	+	++	+++
Control site	—	—	—	—

*Recipient sites were prepared by intradermal injection of 0.1 ml. of the patient's serum and challenged 48 hours later with 0.02 ml. of the insulin preparation (8 units/ml.)

Insulin Precipitating Antibodies.—The patient's serum was tested for precipitins against insulin by two methods. One employed the gel diffusion plate technique with negative results. The other employed the passive cutaneous anaphylaxis technique, modified from the method of Oakley and co-workers.² Serial dilutions of the patient's serum and saline controls were injected intradermally into the shaved abdominal walls of both a guinea pig and rabbit. After 4 hours, a mixture of 0.6 ml. of Evans blue dye, 80 units of regular insulin, and a tracer amount of I¹³¹ labeled insulin‡ was injected intravenously

Ind. *Kindly supplied by Dr. W. R. Kirtley, Eli Lilly Research Laboratories, Indianapolis.

†Hollister-Stier Laboratories, Los Angeles, Calif.

‡Abbott Laboratories, North Chicago, Ill.

into the guinea pig, and a mixture containing twice those amounts was injected into the rabbit. The previously sensitized sites were observed for local accumulation of the dye and were counted at intervals with a collimated scintillation detector to detect local escape of the radioactive tagged insulin. Results of this method also were negative.

Insulin-Binding Antibodies.—The combination of insulin and insulin-binding antibody was demonstrated by two *in vivo* procedures.

The survival time of insulin in the blood was determined after intravenous injection of a tracer dose (30 μ c) of I^{131} tagged zinc insulin. Blood radioactivity was determined at intervals of 3 to 5 minutes, using an intravenous

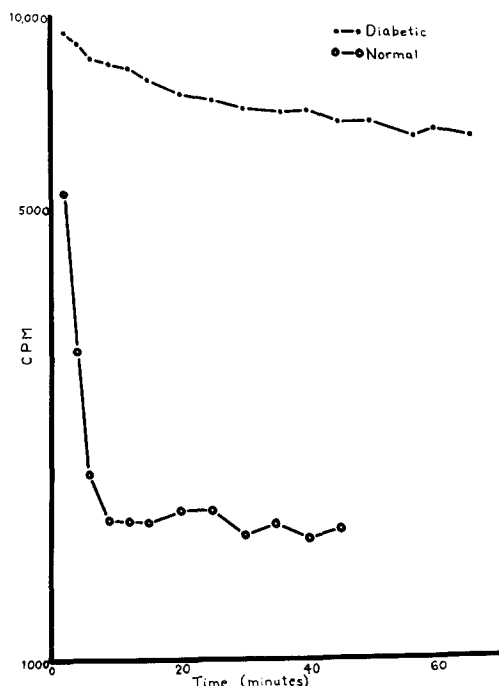


Fig. 2.—Insulin survival curve indicating the rate of disappearance of radioactivity from the bloodstream after intravenous administration of a tracer dose I^{131} labeled insulin. The prolonged intravascular retention of insulin in the insulin-treated diabetic is indicative of insulin binding by antibody.

infusion apparatus connected to a polyethylene coil in a well-scintillation detector. Fig. 2 compares the survival in the present case with that in a non-diabetic individual. The half-time of survival in the patient was 3.5 hr. In the nondiabetic individual there was a rapid decline in blood levels of radioactivity which reached a low stationary level within 10 minutes.

The degree of binding of insulin within the intravascular space was estimated by measuring the apparent volume of distribution of I^{131} labeled insulin (insulin space). This was compared with the plasma volume as determined with radioiodinated serum albumin. In 48 individuals, not previously treated with insulin, the insulin space was 4 to 6 times the plasma volume. In the patient, the insulin space was 3,460 ml. while the plasma volume was 2,850 ml.

In order to correlate the retention of insulin within the blood and the physiologic (hypoglycemic) effect of insulin, an intravenous insulin tolerance test was performed (Fig. 3). Regular insulin was injected intravenously in a dose of 0.1 unit per kilogram body weight. Blood sugar determinations were done by the Folin-Wu method. In contrast to the normal, where a considerable but transient hypoglycemic effect is expected, there was a slow progressive drop in blood sugar level to about 50 per cent of the initial value at the end of the 2 hour test period.

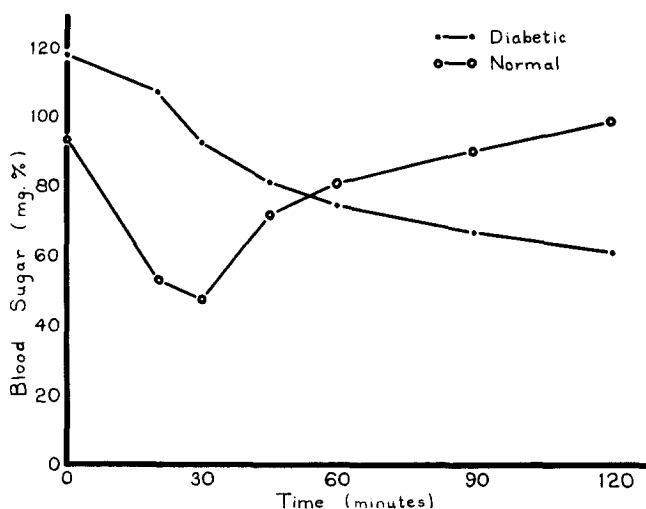


Fig. 3.—Results of intravenous insulin tolerance test. Regular insulin, 0.1 unit per kilogram of body weight, was injected intravenously. The slow, prolonged decline in blood sugar levels in the insulin-treated diabetic is interpreted as indicating a slow release of insulin from insulin-binding antibody.

DISCUSSION

The case described here presents features of both local and generalized urticarial reaction to insulin. Local reactions at the site of insulin injections are relatively common, particularly on the initiation of insulin therapy. They consist of swelling, induration, erythema, and pruritus which usually develops within minutes after injection and subsides within a few hours or days. They are usually mild and, with repeated daily insulin injections, tend to disappear over a period of weeks, apparently as hyposensitization occurs.

In addition to the typical local allergic reaction, this patient demonstrated another type of local skin reaction grossly typical of "lipohypertrophy." This was confined to areas of repeated insulin injections on each thigh. The swelling subsided completely within a week after injections of insulin were given in other sites and did not recur as long as the site was changed daily. The mechanism responsible for this condition is unknown. There is some question as to the effectiveness of insulin injected into an area of lipohypertrophy, since the insulin may be retained in that site rather than released into the circulation.³

Of particular interest in this and in similar cases are attempts to identify the antigen. Intracutaneous tests demonstrated skin sensitizing antibodies to

commercial insulin preparations derived from either beef, pork, or sheep. On the other hand, negative tests resulted from injection of extracts of beef, pork, or sheep protein. These findings are compatible with impressions that allergic reactions to insulin preparations are relatively "insulin specific" rather than "species specific."⁴⁻⁸ There has been little success in using insulin of species other than beef or pork to treat the patient who is allergic to commercial insulin. From a clinical viewpoint, the slight variation in structure of the insulin molecule in purified preparations of insulin from various animal species does not appear antigenically significant since there is considerable cross-reactivity, although of variable degree. It is also possible, although unlikely, that an antigen other than insulin in these preparations might be responsible for the allergic reaction. The antigenicity of endogenous human insulin remains in question.^{7, 9-11}

The degree of response to skin tests employing modified insulin preparations in this patient were roughly correlated with the solubility of the injected insulin. This suggests that protein-bound insulin or insulin crystals may not readily participate in the allergic reaction.

The insulin antibody responsible for skin sensitivity has been characterized to a certain extent, an analogy having been made between insulin allergy and ragweed pollen allergy.^{9, 12} In the allergic individual, manifestations of allergy occur upon interaction of antigen and skin sensitizing antibody (reagin). During the process of hyposensitization, repeated injections of antigen result in increasing titers of blocking antibody. Blocking antibody binds the antigen, making it unavailable for reaction with reagin and consequently decreasing the allergic manifestations. In the allergic patient, the manifestations of allergy depend on the relative titers of reagin and blocking antibody. This concept as applied to insulin antibodies has been used to explain some cases of insulin resistance since insulin bound to blocking antibody apparently is also prevented from exerting its hypoglycemic effect.⁹

Insulin resistance as defined above¹ is relatively rare; however, a state of *relative insulin resistance* may be presumed in those diabetic patients who require greater than physiologic amounts of insulin. A physiologic dose of 50 to 60 units per day is based on the requirement of well-controlled patients with pancreatic diabetes and on the assay studies of Berson and Yalow.¹³ Insulin-binding (blocking?) antibody apparently can be demonstrated in any insulin-treated individual.^{14, 15, 16} The prolonged retention of labeled insulin in the vascular compartment of this patient and the consequent seemingly small volume of distribution of insulin are presumably due to intravascular binding by this antibody.¹⁷ The delayed release of insulin from its antibody bond and its subsequent escape from the intravascular compartment is in turn reflected in the insulin sensitivity test where the rate of drop in blood sugar was slow and the duration prolonged. The binding of insulin is reversible, and the insulin which is released retains its physiologic potency.^{10, 14, 18} Insulin resistance due to insulin-binding antibody implies that only a fraction of the dose of insulin is released from its bond to exert a hypoglycemic effect while much of the bound insulin is permanently inactivated or destroyed.

SUMMARY

A case is presented of a patient who exhibited local and generalized urticarial reactions, presumably due to insulin, associated with increasing insulin requirements and a delayed hypoglycemic response to intravenously administered insulin. The presence of skin-sensitizing antibodies to insulin of various animal species was demonstrated. Insulin-binding (blocking?) antibody was also demonstrated. Administered labeled insulin was largely retained intravascularly, the insulin space approximating the plasma volume. The role of insulin-binding antibody in retaining insulin within the vascular space was related to the diminished physiologic response to insulin. The significance of these immune phenomena in the genesis of frequently observed cases of *relative insulin resistance* is discussed.

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REFERENCES

1. Martin, W. P., Martin, H. E., Lyster, R. W., and Strause, S.: Insulin Resistance: Critical Survey of the Literature With Report of a Case, *J. Clin. Endocrinol.* **1**: 387, 1941.
2. Oakley, W., Field, J. B., Sowton, J. V., Rigby, B., and Cunliffe, A. C.: Action of Prednisone in Insulin-Resistant Diabetes, *Brit. M. J.* **1**: 1601, 1959.
3. Paley, R. G., and Scott, M. H.: Severe Insulin Lipodystrophy as a Possible Cause of Instability in Diabetics, *Brit. M. J.* **2**: 1331, 1958.
4. Lewis, J.: The Antigenic Properties of Insulin, *J. A. M. A.* **108**: 1336, 1937.
5. Wasserman, P., Broh-Kahn, R. H., and Mirsky, I. A.: The Antigenic Property of Insulin, *J. Immunol.* **38**: 213, 1940.
6. Harten, M., and Walzer, M.: Allergy to Insulin, Liver, Pituitary, Pancreas, Estrogens, Enzymes and Similar Substances, *J. ALLERGY* **12**: 72, 1940.
7. Lerman, J.: Insulin Resistance. The Role of Immunity in Its Production, *Am. J. M. Sc.* **207**: 354, 1944.
8. Ezrin, C., and Mañney, P. J.: The Antigenicity of Insulin, *J. Clin. Invest.* **38**: 1002, 1959.
9. Lowell, F. C.: Immunologic Studies in Insulin Resistance. I. Report of a Case Exhibiting Variations in Resistance and Allergy to Insulin, *J. Clin. Invest.* **23**: 225, 1944.
10. Field, J. B., and Woodson, M. L.: Studies on the Circulating Insulin Inhibitor Found in Some Diabetic Patients Exhibiting Chronic Insulin Resistance, *J. Clin. Invest.* **38**: 551, 1959.
11. Arquilla, E. R., and Stavitsky, A. B.: Evidence for the Insulin-Directed Specificity of Rabbit Anti-insulin Serum, *J. Clin. Invest.* **35**: 467, 1956.
12. Loveless, M. H., and Cann, J. R.: Distribution of "Blocking" Antibody in Human Serum Proteins Fractionated by Electrophoresis-Convection, *J. Immunol.* **74**: 329, 1955.
13. Yalow, R. S., and Berson, S. A.: Immunoassay of Endogenous Plasma Insulin in Man, *J. Clin. Invest.* **39**: 1157, 1960.
14. Berson, S. A., Yalow, R. S., Bauman, A., Rothschild, M. A., and Newerly, K.: Insulin-¹²⁵I Metabolism in Human Subjects: Demonstration of Insulin Binding Globulin in the Circulation of Insulin Treated Subjects, *J. Clin. Invest.* **35**: 170, 1956.
15. Arquilla, E. R., and Stavitsky, A. B.: The Production and Identification of Antibodies to Insulin and Their Use in Assaying Insulin, *J. Clin. Invest.* **35**: 458, 1956.
16. Skom, J. H., and Talmage, D. W.: The Role of Nonprecipitating Insulin Antibodies in Diabetes, *J. Clin. Invest.* **37**: 787, 1958.
17. Lennon, E. J., Engbring, N. H., and Engstrom, W. W.: In Vivo Studies of Insulin Transporting Antibody, *Clin. Research* **8**: 242, 1960.
18. Skom, J. H., and Talmage, D. W.: Nonprecipitating Insulin Antibodies, *J. Clin. Invest.* **37**: 783, 1958.