

Insulin allergy and insulin resistance

Case report with immunologic studies

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This case report describes the course of a woman with adult-onset diabetes who received insulin therapy a second time. Shortly thereafter she developed generalized allergic reactions to insulin followed by a period of insulin resistance during which allergic reactions were no longer present. Insulin therapy was stopped, and 7 months later small doses of insulin resulted in generalized urticaria and angioedema. Antibodies to bovine insulin of the IgG, IgA, IgM, and IgE classes and Prausnitz-Küstner antibody activity to bovine insulin were observed in this patient. The findings support the concept of IgE antibody being responsible for the allergic manifestations and IgG antibody producing a protective or "blocking antibody" effect.

The presence of an antibody response to therapeutic heterologous insulin has been known for a number of years.¹ This report describes a patient with adult-onset diabetes mellitus who developed, in sequence, generalized allergic reactions to insulin, rising levels of circulating antibodies to bovine insulin, and insulin resistance. The generalized allergic reactions to insulin disappeared during the period in which insulin requirements were high, only to reappear later.

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The antibodies to bovine insulin contained in serial serum specimens were examined and considered in relation to the clinical course. Antibodies to bovine insulin were observed in the IgG, IgA, IgM, and IgE classes. The finding supports the concept of IgE antibody being responsible for the allergic manifestations and IgG antibody producing a protective or "blocking antibody" effect.

CASE REPORT

A 56-year-old woman (A. P. Unit No. 350995) was admitted to the Buffalo General Hospital for control of diabetes mellitus which had been diagnosed 6 years before and treated for 4 months with NPH insulin followed by chlorpropamide. There had been no hypoglycemic therapy for at least one year prior to this admission. Symptoms on admission included those of vaginitis, migratory paresthesias, urinary frequency, dysuria, nocturia, and blurred vision.

The past history included hypertension, cholecystectomy, removal of a benign mass from the neck, and one episode of pneumonia. The allergic history was negative except for a history of itching sensation after eating tomatoes and strawberries.

The family history was positive for diabetes; the patient's father, brother, and one of her 5 children had diabetes mellitus. Except for the fact that one daughter developed hives after ingestion of tomatoes, there was no family history of allergy.

Physical examination revealed an obese woman of 208 pounds, blood pressure 130/70, pulse 86, temperature normal, and respirations 16 per minute. Perineal intertrigo, vulvovaginitis, and cervicitis were present. There was mild pitting edema of the lower legs. Vibration and position senses were diminished in the lower extremities, and the Achilles reflexes were absent.

Results of laboratory examinations were: Urinalysis—protein trace, glucose 4+, white blood cells per high-power field 15 to 20, red blood cells per high-power field occasional, and specific gravity 1.033; hematocrit 41 per cent; hemoglobin 12.5; white blood cells 7,500 with 12 per cent bands, 45 per cent filaments, 4 per cent eosinophils, 34 per cent lymphocytes, and 5 per cent monocytes; blood urea nitrogen 14 mg. per 100 ml.; cholesterol 328 mg. per 100 ml.; fasting blood glucose 324 mg. per 100 ml. *B. aerogenes* was cultured from the urine. An intravenous glucose tolerance test showed a low normal disappearance of glucose ($K_G = 1.2$) and an abnormal free fatty acid response characteristic of diabetes mellitus.

The vulvovaginitis was treated with mycostatin vaginal suppositories. The diabetes was controlled with a 1,500 calorie diet (150 Gm. carbohydrate, 80 Gm. protein, and 70 Gm. fat) and 32 units of NPH insulin. Mild redness and swelling at the site of insulin injections were noted by the patient and nursing staff prior to discharge from the hospital on Day 12 of insulin therapy (Fig. 1).

Six or 8 hours after discharge a generalized rash and facial swelling were noted by the patient. On Day 14 she came to the emergency room with generalized urticaria and angioedema of the lips and eyes. Lente insulin was substituted for NPH insulin, and Benadryl, 50 mg., was injected intramuscularly to be followed by 50 mg. orally 3 times a day. The following day a digestion blood sugar was 388 mg. per 100 ml., and the urine was strongly positive for acetone. Signs of the allergic reaction were receding, and lente insulin was continued in the dosage of 32 units per day. The next day there was vomiting. The insulin dosage was omitted, and after 17 days of insulin therapy the patient was admitted to the hospital with dehydration and ketoacidosis.

Physical examination revealed a blood pressure of 170/80, pulse 106, respirations 28, temperature 97° (orally), and a fruity odor to the breath. The patient was mentally clear, flushed, had a dry mouth, and the abdomen was distended and tympanitic. Blood glucose was 696 mg. per 100 ml., blood urea nitrogen 31 mg. per 100 ml., and sodium 134 mEq. per liter. The serum was strongly positive for acetone, and the plasma bicarbonate was 5.2 mEq. per liter. The white blood cell count was 27,000 with 1 per cent myelocyte, 41 per cent bands, 41 per cent filaments, 10 per cent lymphocytes, and 7 per cent monocytes.

In the 24 hours after admission 2,000 units of crystalline zinc insulin were administered to control the diabetic ketoacidosis. Intake included 9,500 ml. of fluids, 1,632 mEq. of sodium, 638 mEq. of bicarbonate, and 300 mEq. of potassium. Fluid output was 6,900 ml. After 24 hours the blood glucose was 184 mg. per 100 ml., the serum bicarbonate was 19.8 mEq. per liter, and a trace of acetone was detected in an undiluted specimen of serum. A throat swab was taken on admission, and the culture grew beta hemolytic streptococci. Erythromycin, 200 mg. every 6 hours, was begun.

Signs of the allergic reactions were absent following the first day in the hospital. After 10 days in the hospital the insulin requirement was 100 units per day. The hospital course was complicated by infection at a cut-down site which became apparent on the third hospital day and progressed to septicemia. A 28 day course of therapy included intravenous benzylpenicillin and methicillin followed by oral oxacillin. An erythematous papular and petechial rash appeared on the twentieth hospital day and gradually diminished and disappeared over a period of 10 days. Skin tests with benzylpenicillin, benzylpenicilloyl-polylysine, and methicillin prior to the penicillin therapy and with these agents and oxacillin following the therapy were all negative.

There was a progressive decrease in insulin requirements, and after 2 months in the hospital the patient was discharged while receiving an all beef lente insulin preparation in a dose of 30 units per day. The diabetes was well controlled, but there were mild local reactions to insulin. Approximately 4 months after the onset of insulin therapy, insulin was discontinued. Seven months later an abscess developed on the arm. A self-prescribed dose of 6 units of insulin subcutaneously resulted in generalized urticaria. One month later a skin test for insulin sensitivity resulted in a generalized urticarial reaction.

The patient continues to be followed at the time of this writing in July, 1969, and is currently controlled on tolbutamide, 1 Gm. twice a day.

IMMUNOLOGICAL STUDIES

Methods and materials

Direct skin tests and Prausnitz-Küstner (P-K) tests. Direct intradermal skin tests for the presence of wheal and flare reactivity to bovine insulin were performed with 0.02 ml. of crystalline zinc insulin in a concentration of 8 units per milliliter. For P-K tests serum specimens from Patient A. P. were serially diluted in sterile physiological buffer. One-twentieth milliliter of each dilution was injected intradermally to sensitize skin sites of 2 nonallergic volunteer recipients. These sensitized sites were challenged 24 hours later with 0.02 ml. of crystalline zinc insulin (8 units per milliliter).

Antisera. Commercial goat antisera to human IgG, IgA, and IgM were used.* Antiserum to IgA was exhaustively adsorbed with 10 times concentrated cord serum. An antiserum to human IgE,† prepared in rabbits, was utilized.

Labeled antigens. Bovine insulin‡ was separated from the contained zinc by three cycles of isoelectric precipitation. This insulin preparation and a purified ragweed antigen, antigen E,§ were labeled by the chloramine T method,¹³ passed through Sephadex G-25|| and stored in the frozen state until used.

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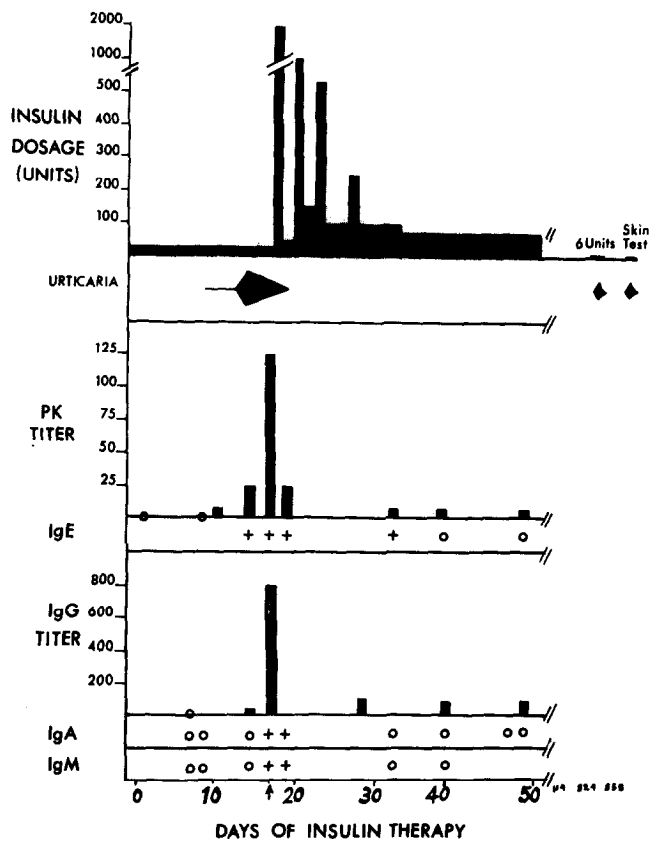


Fig. 1

Daily insulin dosage, the course of allergic reactions to insulin, and antibodies to bovine insulin are plotted. The arrow at Day 17 indicates the time of admission to hospital.

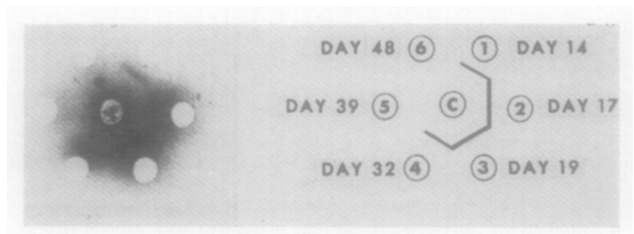


Fig. 2

IgE antibody to bovine insulin. Radioimmunoassay examination of serum for IgE antibody to bovine insulin: A photographic representation of the radioautograph is on the left, and an explanatory diagram on the right. Antiserum to human IgE and I^{125} -labeled bovine insulin was placed in the center well (C). Serial serum specimens from Patient A. P. were placed in peripheral wells 1 to 6. There was no visible precipitate. The radiograph demonstrates the presence of IgE antibody to bovine insulin on Days 14, 17, 19, and 32 of therapy.

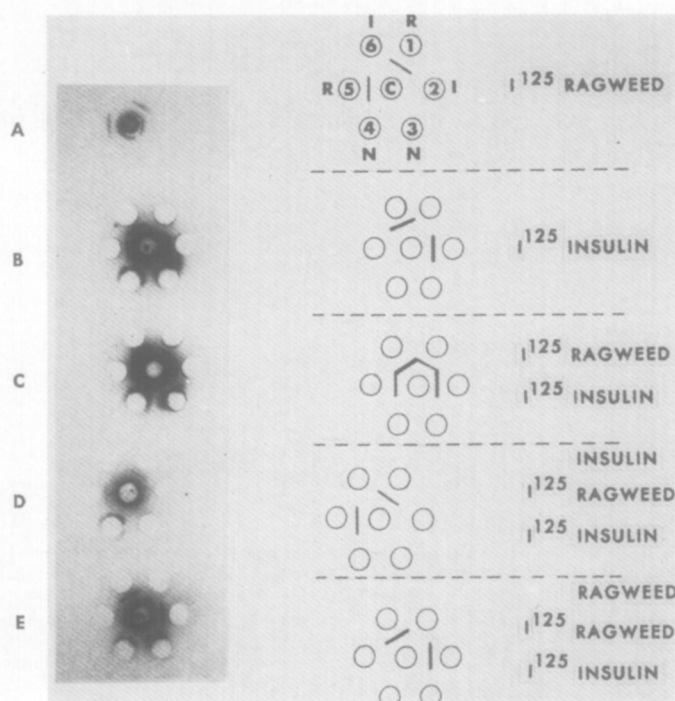
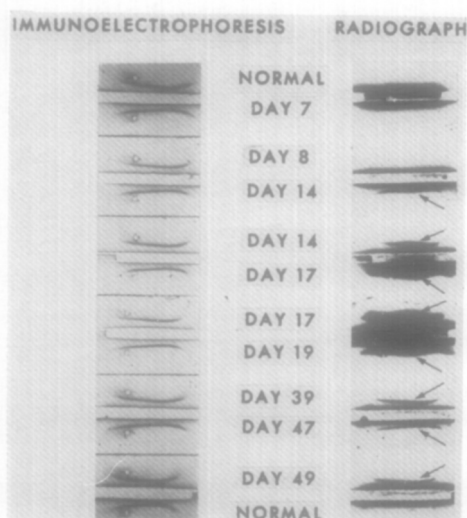


Fig. 3

IgE antibody to bovine insulin and ragweed antigen E. Radioimmunodiffusion comparison of IgE antibody to ragweed antigen E and antibody to bovine insulin: A photographic representation of the radiograph is on the left, and explanatory diagram on the right. Antiserum to human IgE was placed in the center wells (c) of tests A to E. In addition, the agents listed on the right were serially placed in corresponding center wells. Contents of the peripheral wells 1 to 6 were the same in tests A to E. Peripheral wells 1 and 5, serum of ragweed-allergic Subject D. A. (R); wells 2 and 6, serum of insulin-allergic Subject A. P. (I); wells 3 and 4, serum of a normal subject (N). Tests A and B illustrate that the I^{125} -labeled antigens were bound only by sera from patients with corresponding allergies. In test C there is a line of complete fusion between the labeled precipitates formed by the ragweed serum and insulin serum demonstrating the similarity of the antibodies to the two agents. Tests D and E confirm antibody specificity of binding. There is an inhibition by nonlabeled antigen of the binding of corresponding labeled antigen.

Radioimmunodiffusion (RID) and radioimmuno-electrophoresis (RIEP). Immunoelectrophoresis and immunodiffusion were performed on microscopic slides in an LKB slide carrier. Following the development of globulin-antiglobulin precipitin lines, slides were washed for 24 hours in isotonic borate buffer, pH 8.0, and labeled antigen was then added to the center well (RID) or trough (RIEP). In some experiments nonlabeled antigen was introduced in excess, allowed to diffuse into the agar, and then labeled antigen was added. Twenty-four hours later a 3 day washing of the slides in three changes of borate buffer was begun. Slides were then dried and exposed to Kodak industrial x-ray film type KK for a period varying from 3 days to 4 weeks. Following removal of the radiographic film, slides were stained with amido black.

**Fig. 4**

IgG antibody to bovine insulin. Radioimmuno-electrophoretic demonstration of antibody to bovine insulin: The IgG precipitation arcs are illustrated on the left, and the corresponding radiograph on the right. The centrally placed labels indicate the wells in which normal sera and serial serum specimens of Patient A. P. were placed for electrophoresis. Antiserum to human IgG and I^{125} -labeled bovine insulin were placed in each trough. The arrows indicate the binding of the label to IgG arcs. Antibody to bovine insulin of the IgG class was present on Days 14, 17, 19, 39, 47, and 49 of therapy. The appearance of the radioactive arcs provide an indication of the relative levels of antibody activity (maximal on Days 17 and 19).

RESULTS

Immunological findings are summarized in relation to the clinical course in Fig. 1.

Direct skin tests

Direct skin tests with crystalline zinc insulin were positive on each occasion they were done. Skin tests were performed shortly after the onset of ketoacidosis, on the thirty-eighth day of therapy, and 8 months after insulin therapy was discontinued. The latter resulted in a generalized urticarial rash within one hour.

Prausnitz-Küstner (P-K) tests

There was an increased P-K titer of the serum on Day 14 and a maximum titer was recorded on Day 17 at the time of admission to the hospital with diabetic ketoacidosis (Fig. 1).

In vitro antibody studies

A radioimmuno-diffusion test for the presence of IgE antibodies to bovine insulin is illustrated in Fig. 2. Immunodiffusion reactions between antiserum to IgE and human sera failed to produce visible lines of precipitation due to the low concentrations of IgE in the serum. The radiograph, however, reveals

binding of I¹²⁵-labeled bovine insulin to the IgE antibody in the patient's serum on Days 14, 17, 19, and 32.

Fig. 3 demonstrates the specificity of this binding and the relationship of these antibodies to those occurring naturally in the serum of a patient allergic to ragweed pollen. Radioimmunodiffusion examination of the serum of a ragweed-allergic subject (D. A.) revealed no detectable IgA or IgM and barely detectable IgG antibody to ragweed. The P-K titer of this serum to ragweed was 1:810. As with the serum of the patient described in this report, IgE anti-serum did not produce visible lines of precipitation with serum from the ragweed-allergic subject, but radioautography demonstrated binding of labeled antigen to antibody. There was also a line of complete fusion between the radioactive lines formed by this serum with antibody to ragweed and the serum with antibody to insulin (Patient A. P.), demonstrating a similarity of the two types of antibodies (Fig. 3, C). The antibody specificity of the binding of labeled antigen is confirmed by a selective inhibition produced by corresponding nonlabeled antigen (Fig. 3, D and E).

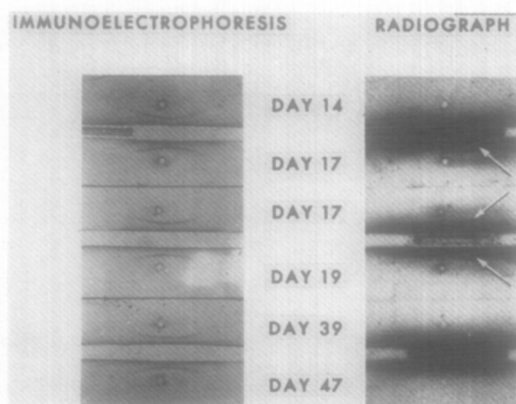
A quantitative comparison of various serum specimens from Patient A. P. was obtained by serial dilution of serum from the insulin-allergic patient (A. P.) in normal serum followed by radioimmunodiffusion examination for the IgG antibodies to insulin. IgG antibody activity was not detectable in the sera of Day 7 and Day 8. Detectable antibody was present in A. P. serum in a dilution of 1:30 on Day 14, 1:810 on Day 17, 1:90 on Day 39, and 1:90 on Day 49 (Fig. 1). A radioimmuno-electrophoretic demonstration of IgG antibodies to insulin in undiluted serum specimens is illustrated in Fig. 4. IgG antibody to bovine insulin was increased prior to the detection of insulin resistance and the increase in the insulin dosage, and it continued to be present in subsequent serum specimens examined.

Serum IgA and IgM antibodies to bovine insulin were detectable only on Day 17 and Day 19 (Figs. 5 and 6).

DISCUSSION

Therapeutic preparations of insulin generally contain mixtures of bovine and porcine insulin and are antigenic in man. Circulating antibodies to insulin are present within a few months after the onset of insulin therapy.¹ However the insulin-binding capacity of the serum remains low in most diabetic persons.² The potential functional significance of antibodies to insulin is indicated by the demonstration of complexes of insulin with antibodies in the sera of insulin-treated diabetic patients¹ and the inhibition by antibodies of insulin-induced hypoglycemia in animals.³⁻⁶ Insulin-resistant states are generally associated with high serum levels of insulin-binding antibodies,^{2, 7} and there is often a therapeutic advantage in the use of insulin preparations with low antigenicity in man.⁸⁻¹⁰

Insulin-resistant diabetes may be present with or without associated insulin allergy.¹¹ Lowell^{3, 4} reported the course of a patient in whom allergy to bovine insulin alternated with insulin resistance. Serum levels of plasma Prausnitz-Küstner (reaginic, skin-sensitizing) and insulin-neutralizing antibody activity

**Fig. 5**

IgA antibody to bovine insulin. Radioimmuno-electrophoretic demonstration of IgA antibody to bovine insulin: Wells were filled with Patient A. P. sera of the days indicated. Troughs contained antiserum to human IgA and I^{125} bovine insulin. The arrows indicate the presence of IgA antibody to insulin on Days 17 and 19 of therapy.

varied independently of each other, and the latter were high during periods of insulin resistance.^{3, 4} A diabetic patient studied by Loveless¹² received prolonged cautious administration of insulin despite the presence of allergy to insulin. Reactions to insulin ceased during the course of therapy, but after a 2 month lapse in therapy the administration of insulin resulted in a systemic allergic reaction. At the time of these reports it was not possible to identify the immunoglobulin classes of antibodies to insulin, but more recently circulating antibodies to insulin of the IgG, IgA, and IgM classes have been observed in the sera of diabetics.¹³ It is now possible to relate many of the biological properties of antibodies to the immunoglobulin classes to which they belong. For example, the skin-sensitizing or Prausnitz-Küstner antibody activity present in the serum of naturally allergic subjects is now known to be a function of antibody of the IgE class.¹⁴ "Blocking antibody" activity, which is present in the sera of naturally allergic subjects after the injection of antigen and limits the interaction of antigen with Prausnitz-Küstner antibody, is largely of the IgG class.¹⁵

The diabetes in the subject described was controlled for a long period without insulin therapy. Shortly after the onset of renewed insulin therapy there were generalized allergic reactions to insulin followed by a period of increased requirements for insulin during which the generalized allergic reactions disappeared. After the insulin therapy was discontinued she again developed generalized allergic reactions from administered insulin. Between the ninth and seventeenth day of insulin therapy there was an appearance of IgG, IgA, IgM, IgE, and P-K antibodies to insulin in the serum. This corresponded to the time period in which there were allergic reactions and then the development of insulin resistance. The rising antibody levels preceded the use of high doses of insulin and therefore did not represent a response to an unusually large antigenic stimulus. There is no clear explanation for the antibody response

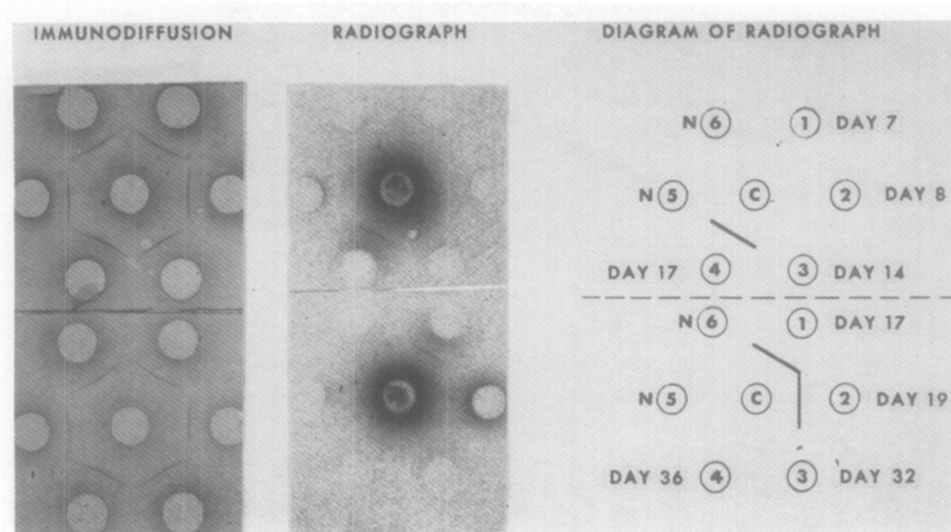


Fig. 6

IgM antibody to bovine insulin. Radioimmunoassay demonstration of IgM antibody to bovine insulin: The immunodiffusion pattern, the corresponding radiograph, and a diagram of the radiograph are shown. Antiserum to human IgM and I^{125} -labeled bovine insulin were placed in the center wells labeled C. Peripheral wells 1 to 4 and 7 to 10 contained serial specimens of Patient A. P. serum. Normal sera were placed in the wells labeled N. The lines of precipitation between the sera and anti-IgM can be seen in the immunodiffusion pattern. The radiograph and corresponding diagram demonstrate the presence of IgM antibody to bovine insulin on Days 17 and 19 of therapy.

which included the various classes of immunoglobulins. The possible relation of this broad spectrum of response to the presence of allergy, to ketoacidosis, or to the presence of infection remains speculative. Murthy and colleagues¹⁶ have observed a relationship between the magnitude of antibody response to insulin and allergic reactions: The sera of diabetic children with local skin reactions to insulin bound greater quantities of insulin than the sera of diabetic children with no reactions to insulin.

The observed rise and fall of serum P-K activity and IgE antibody in association with corresponding changes in antibodies to insulin of the other immunoglobulin classes raises the question of a possible general relationship of IgE to the immune response in man. The existence of this immunoglobulin class in human serum and its relationship to the Prausnitz-Küstner antibody activity characteristic of allergic states have been demonstrated by the studies of Ishizaka and co-workers.^{17, 18} Their findings have been supported by recent studies with the use of a myeloma protein of the IgE class.¹⁹⁻²¹ IgE is present in normal serum, although in small quantities,²² and may have a normal function in protection from infectious microorganisms.²³ Serum levels of IgE may be increased in numerous disorders including allergic states.^{22, 24, 25}

The Prausnitz-Küstner antibody which appears during serum sickness reactions resulting from immunization has been reported to be associated with immunoglobulin classes other than IgE.²⁶ Findings in the patient reported here

demonstrate that antibodies of the IgE class may be present in an allergic state resulting from insulin therapy. The observation of a line of complete fusion on radioimmunodiffusion analysis between antibodies to ragweed and insulin (Fig. 3, C) illustrates the antigenic identity of these antibodies. Patterson and co-workers²⁷ have recently reported findings which indicate that the reaginic antibody of an insulin-allergic subject was in an immunoglobulin class other than IgG, IgA, and IgM.

During the period between Day 17 of insulin therapy and the termination of therapy approximately 3 months later there was an absence of generalized allergic reactions to insulin. A possible exception was a transient, unexplained erythematous-papular-petechial eruption. The serum contained P-K antibody to bovine insulin during this period, and IgE antibody to insulin was demonstrable on Days 14, 17, 19, and 32. Moreover, direct skin tests with insulin continued to produce a wheal and flare response. It is therefore considered unlikely that the absence of generalized allergic reactions to insulin during this period was due to an absence of available IgE antibody. The absence of reactions was probably due, at least in part, to a lack of available antigen. In the patient reported by Lowell,^{3, 4} symptoms of allergy decreased during the course of insulin therapy in the absence of a change in serum levels of P-K antibody. Insulin-neutralizing antibody appeared in the serum during therapy, and a possible protective effect of this antibody was considered. Tennenbaum and co-workers²⁸ have provided evidence for this type of effect in experimental studies in dogs. Normal dogs, passively sensitized with the serum from dogs naturally allergic to ragweed, developed anaphylactic reactions following the intravenous administration of antigen. The additional infusion of serum from normal dogs hyperimmunized with ragweed antigen prevented the anaphylactic reactions. Levine and co-workers^{29, 30} have examined the determining factors in allergic reaction to penicillin. In patients with wheal and flare skin reactivity to benzylpenicilloyl-polylysine (BPO), they found that serum IgG BPO-specific antibodies were also present. Upon the therapeutic administration of penicillin, allergic reactions began only when the IgG antibody titers were considerably reduced and ceased when IgG antibody titers rose above the original levels. The penicillin-allergic patient with the highest titer of circulating IgG BPO-specific antibodies failed to develop an allergic reaction. In the diabetic patient reported here, the maximum observed level of IgG antibody was present on Day 17 of therapy. IgG antibody to insulin continued to be present during the course of therapy, whereas IgA and IgM antibody to insulin were not detectable in the sera tested after Day 19. Thus a protective or "blocking antibody" function could be attributed only to antibody to insulin of the IgG class.

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