Insulin Resistant Diabetes with Insulin Antibodies

A Case Report

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Diabetes is considered to be insulin resistant when the patient fails to respond to ordinary doses of insulin.[1] Insulin resistance may occur in infection, liver disease, hyperthyroidism, hyperadrenocorticism, and hyperfunction of the anterior lobe of the pituitary. Lerman[2] was the first to postulate, and Lowell[3] the first to demonstrate, insulin neutralizing antibodies in the serum of insulin resistant diabetics. The importance of immune reactions in insulin resistance was emphasized by Lukens.[4]

Goldner and Ricketts[5] noted the association of insulin refractoriness with insulin allergy while Root[6] related both phenomena to the occurrence of antibodies against insulin. Lowell[7] related the allergic insulin reactions to the presence of skin sensitizing antibodies, different from insulin neutralizing antibodies.

The present study is concerned with a case of insulin resistant diabetes in which both insulin blocking antibodies and skin sensitizing antibodies could be demonstrated in the serum.

REPORT OF CASE

N. K., a 49-year-old woman known to have had diabetes since 1953, was admitted to the medical department in February 1955.

After a short febrile disease her diabetes became more severe, the fasting blood sugar reaching 380 mg. per 100 ml., with 10 per cent glucose in the urine and ketonuria. She had received regular and protamine-zinc insulin with interruptions since the diabetes had been diagnosed. Her past history revealed no allergic phenomena, and she had not received blood transfusions.

Physical examination was essentially normal. The temperature was normal, the blood pressure was 150/90 mm. Hg. Gynecologic, ophthalmologic, and neurologic examinations did not reveal abnormal findings. On admission the urine contained a large amount of glucose and acetone; there was no albuminuria, the sediment was normal and a urine culture was negative. Blood examination showed normal hemoglobin value and cell counts and a normal sedimentation rate. The fasting blood sugar was 280 mg. per 100 ml., urea 48 mg. per 100 ml., cholesterol 300 mg. per 100 ml., total serum protein 6.73 gm. per 100 ml., albumin 3.70 gm. per 100 ml., globulin 3.03 gm. per 100 ml.; routine liver function tests were normal. Blood cultures were negative.

Urinalysis showed normal values for 17-ketosteroids (6.8 mg.), 11-oxysteroids (1.45 mg.), estrogens (25 units) and FSH (100 units) per 24 hours. The basal metabolic rate was plus 20 per cent. Roentgen examinations of the skull, sella turcica, and gastrointestinal tract, cholecystography and intravenous pyelography gave normal results. Electroencephalography (repeated eight times) constantly showed remarkable bilateral, mainly frontal disturbances, slightly accentuated on the left side.

Course in hospital

During the first few days after admission the patient was treated with 60 units of regular insulin per day and a diet containing 50 gm. of carbohydrate, 70 gm. of protein and 70 gm. of fat. On this regime she excreted 70 gm. of sugar per day. The daily insulin dose was increased gradually to a maximum of 850 units. Even with such a dose, hypoglycemia did not occur; the daily urinary sugar varied from 16.0 to 102 gm. and the blood sugar from 220 to 514 mg. per 100 ml. There was no difference in glucose excretion whether local insulin (Galma) or imported (Boots or Organon) was used.

A 24-hour interruption of insulin treatment, necessary

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for the performance of the Lowell mouse test, caused the blood sugar to rise to 560 mg. per 100 ml.; acetone appeared in the urine, and 170 gm. of glucose was excreted in twenty-four hours.

When the administration of 850 units per day was resumed, blood sugar values dropped to 231 mg. per 100 ml., and the daily glucose excretion to 30-40 gm. after about one week. Acetonuria disappeared within three days.

Intravenous infusions of ACTH, 10 mg. daily for two weeks, caused marked improvement, and insulin could be reduced to 430 units per day. On further reduction of insulin, however, sugar excretion increased.

During a subsequent period of two weeks BAL (dimercaptopropanol) was administered intramuscularly, 100 mg. twice per day, while ACTH was discontinued. During this period insulin could be reduced to 200 units per day without increase of sugar excretion.

In July 1955, the patient was discharged from the hospital in satisfactory condition with 300 units of insulin daily and a diet consisting of 150 gm. of carbohydrate, 70 gm. of protein and 70 gm. of fat.

The patient was re-hospitalized twice during 1955, once in August after a short upper respiratory infection which caused aggravation of the diabetes. On admission 75 gm. glucose and a positive acetone reaction were found in the twenty-four hour urine collection. After administration of 1,500 units of insulin within the first eighteen hours, acetone disappeared from the urine and glucose excretion dropped to 20 gm. per day. During the next twenty-one days, 500-620 units of insulin per day were administered, and the patient was discharged after forty-four days with 300 units of insulin per day, excreting 5 gm. of sugar in the urine per day, which was free of acetone.

On the second admission, in October 1955, glucose excretion amounted to 40 gm. per day and acetone was again present, the increase occurring without previous infection. Daily administration of 600 to 700 units of insulin, combined with infusions of 10 mg. of ACTH during fourteen days, caused marked improvement. Subsequently cortisone was given, 200 mg. daily for six days and then in gradually diminishing doses to 25 mg. per day.

On discharge the patient excreted 0 to 2.5 gm. glucose per day and had no acetone in the urine. On the day of discharge the fasting blood sugar was 246 mg. per 100 ml. She was instructed to take 300 units of insulin daily and 25 mg. of cortisone every second day and a diet as before.

SPECIAL INVESTIGATIONS

a. *Glucose and insulin tolerance tests*

Fasting blood sugar levels were determined at thirty-minute intervals for three hours after the intravenous injection of 20, 40, or 60 units of regular insulin. Figure 1 shows that the blood sugar values decreased only slightly, the decrease in the first half hour being less than 50 per cent.[8]

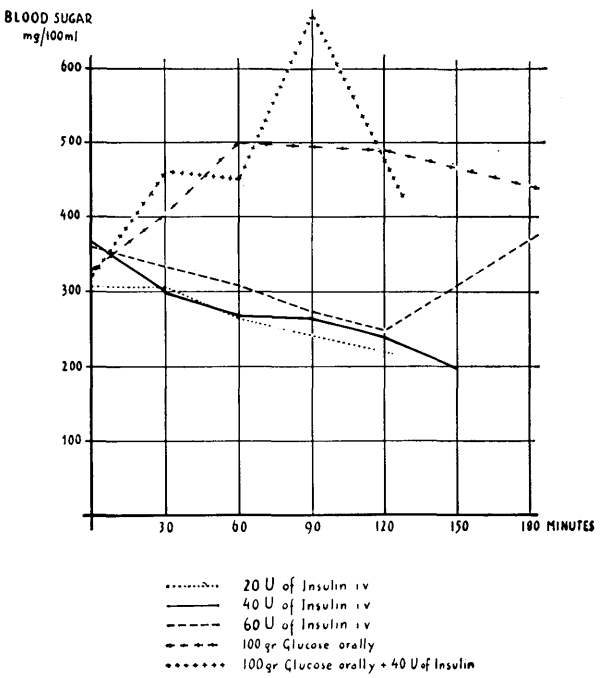

FIG. 1. Glucose and insulin tolerance tests.

In addition, a glucose-insulin tolerance test was carried out. Blood sugar values were determined just before and at thirty-minute intervals for three hours after the simultaneous administration of 100 gm. of glucose (orally) and 40 units of insulin intravenously. The resulting blood sugar values, compared with those obtained after the oral administration of 100 gm. glucose without insulin, show that insulin had no effect on the blood sugar.

b. *Lowell mouse test*

The Lowell mouse test was performed according to Eskind, Franklin and Lowell.[9] The ED_{100} of local insulin (*Galma*) (*smallest quantity of insulin producing hypoglycemic* signs such as paralysis of hindlegs and convulsions in 100 per cent of the animals tested) for 20 gm. mice was found to be 0.1 unit.

Mixtures of 0.1 unit of insulin with varying amounts (0.25-1.5 ml.) of normal human serum, of the patient's serum, and of physiologic saline were prepared. Suffi-

cient glucose was added to the normal serum and to the saline to produce glucose concentrations equal to those in the patient's serum. The mixtures were incubated at 37° for one hour and injected intraperitoneally.

Table 1 shows that 1.5 ml. of the patient's serum protected the animals completely from the hypoglycemic action of insulin. With decreasing amounts of the patient's serum the protective effect diminished.

TABLE 1

Lowell mouse test
Intraperitoneal injection of 0.1 U of insulin, preincubated with serum

Mixture ml. injected	No. of mice showing hypoglycemic signs per total injected: Patient's serum + insulin	Normal serum + insulin	Saline + insulin
1.5	0/5	5/5	5/5
1	1/5	5/5	5/5
0.75	2/5	4/5	5/5
0.50	4/5	5/5	5/5
0.25	5/5	5/5	5/5

c. *Rat diaphragm glucose uptake and glycogen synthesis*

The inhibitory effect of the patient's serum on the enhancing influence of insulin on glucose utilization and glycogen synthesis by rat diaphragm was tested by the method of Gemmill.[10]* The action of insulin after incubation with the patient's serum, was compared with the action of insulin incubated with normal serum to which glucose had been added to match the concentration in the serum of the patient.

The addition of 0.1 unit of insulin to a rat diaphragm preparation evoked more than twofold increase of glucose utilization (table 2). Previous incubation of insulin with the patient's serum abolished this effect.

Glycogen synthesis by rat diaphragm, incubated with normal human serum, was greatly enhanced by the addition of 0.025 unit of insulin. In the presence of the patient's serum this amount of insulin had no such effect. Addition of 0.1 unit of insulin, however, produced increased glycogen synthesis even after incubation with patient's serum.

d. *Demonstration of antibodies to insulin by red cell agglutination*

Sheep red blood cells were treated with a 1:20,000 tannic acid solution, and insulin was conjugated to the red cells by the method of Stavitsky and Arquilla.[11, 12] When the undiluted patient's serum or a 1:1 dilution

*The diaphragm tests were kindly carried out by Prof. E. Wertheimer, Department of Biochemistry, The Hebrew University Medical School, Jerusalem.

TABLE 2

Rat diaphragm test

Insulin units	Glucose utilization mg./100 mg. diaphragm: Normal serum	Patient's serum	Glycogen synthesis mg./100 mg. diaphragm: Normal serum	Patient's serum
0.	0.53	0.87	0.070	0.030
0.025	—	—	0.124	0.036
0.1	1.19	0.92	0.190	0.140

Glucose concentration patient's serum: 380 mg. per 100 ml.

Glucose concentration normal serum: 100 mg. per 100 ml.

plus additional 280 mg. per 100 ml. = 380 mg. per 100 ml.

of this serum in physiologic saline was added to such treated red cells, hemagglutination resulted, demonstrating the presence of insulin antibodies. Control experiments with normal serum and the patient's serum with untreated red cells were negative.

e. *Skin sensitizing antibodies*

Active and passive transfer skin tests were performed. In the active skin test a quantity of 0.1 units of insulin was injected intradermally and the whealing effect compared with that of a control injection of physiologic saline solution. A positive reaction to insulin was observed (table 3). Similar injections of insulin did not produce any whealing effects in normal persons.

A passive transfer test, performed according to Prausnitz-Küstner by injecting insulin intradermally into a normal person who had received an intradermal injection of the patient's serum at the same site twenty-four hours previously, was strongly positive (table 3). Controls with normal serum were negative.

f. *Electrophoresis of patient's serum*

Electrophoresis on filter paper of the serum of the patient was carried out on Whatman No. 1 filter paper,

TABLE 3

Skin sensitizing antibodies

Substances injected intracutaneously	Active skin sensitivity test: Patient	Normal	Passive transfer test: Normal
Insulin	+++	—	—
Saline	—	—	—
Insulin 24 hrs. after patient's serum			+++
Insulin 24 hrs. after normal serum			—

— no wheal produced.

+++ wheal of 2 cm. diameter produced after 20 min.

in a veronal-Na acetate buffer, *p*H 8.6, ionic strength 0.1, duration sixteen hours, current 4 volt/cm., staining with amidoblack 10 B. No increase of gamma globulin was found (19.2 per cent of total serum protein, normal range 13-25 per cent). The β-globulin value was slightly increased (18.2 per cent, normal range 10.5-16.3), while α_2 globulin was decreased (5.4 per cent, normal range 9.0-15 per cent).

DISCUSSION

The insulin resistance in this patient could be related to the presence of an insulin antibody in her serum. No disturbances of thyroid, adrenal, anterior pituitary, or liver functions were found, and although the patient suffered from repeated infections the insulin resistance persisted after their cure. Electroencephalographic disturbances have been described in cases of unstable diabetes.[13] Although aberrations of the electroencephalogram had been observed repeatedly in the present patient, their possible relationship to the insulin resistance has not been established.

The presence of an insulin neutralizing factor, already suggested by the weak response to insulin in the tolerance tests, was proved by the Lowell mouse test.

The rat diaphragm test performed with the patient's serum showed an inhibitory effect on insulin action similar to the findings of Marsh and Haugaard[14] who examined the serum of an insulin resistant diabetic. However, the insulin inhibitory action of the serum of our patient could be demonstrated only when relatively small quantities of insulin were applied in the test and could be overcome by adding more insulin.

Lowell[7] suggested that the insulin neutralizing activity of insulin resistant diabetics, as demonstrated with the mouse test, is due to insulin neutralizing antibodies. Evidence for the presence of insulin antibodies in the serum of the present case was obtained by the positive active and passive transfer skin tests and by the agglutination of insulin-sensitized tannic acid treated sheep erythrocytes.[11, 12] Infection and intermittent use of insulin, both considered conducive to the development of insulin antibodies,[6, 18] may have been factors in the production of insulin resistance in our patient. There is no proof, however, that the insulin neutralizing factor and the insulin antibodies, as demonstrated in the skin and hemagglutination tests, are identical.

Jorpes[15] doubted the existence of skin sensitizing insulin antibodies in cases of insulin allergy, as he successfully prevented allergic reactions to insulin in a hypersensitive patient by the use of a recrystallized commercial insulin preparation. Loveless and Cann[16] demonstrated that the skin sensitizing insulin antibodies and the insulin neutralizing serum factor are present in different serum protein fractions, the former in the beta globulin fraction, the latter closely bound to the gamma globulin fraction. Sehon and co-workers,[17] using zone electrophoresis, reported an increase of serum gamma globulin in a case of insulin resistant diabetes. In our patient whose serum contained both skin sensitizing insulin antibody and insulin neutralizing factor, electrophoretic examination showed normal gamma globulin. The slightly increased beta globulin value might be due to high cholesterol content.

When very high doses of insulin were administered to our patient, ketonuria disappeared and glucose excretion decreased. Parallel to this were the results of the rat diaphragm test in which large doses of insulin overcame the insulin inhibitory effect of serum. It may be assumed that part of the insulin administered was engaged in the inactivation of the insulin neutralizing factor, the remainder of the insulin manifesting its normal action on carbohydrate metabolism. The results of the mouse test are in agreement with such a supposition. Inhibition of the action of insulin was observed as long as a sufficient quantity of serum was used.

As to the value of the use of corticotropin or corticosteroids in the treatment of insulin resistant diabetes, opposite results have been reported. Loveless and Cann[16] and Kaye, McGarry and Rosenfeld[18] reported that corticotropin administration markedly aggravated the diabetic state, whether given during a period of relatively low insulin requirement (160 units per day), or of very high requirement (900-1100 units per day). Our results, on the other hand, are in agreement with those of Howard,[19] Gitelson,[20] and Kleeberg, Diengott and Gottfried[21] who obtained improvement with ACTH.

It is known that alloxan induced experimental diabetes is accompanied by a temporary fall of blood glutathione,[22, 23] and that alloxan diabetes can be prevented by the administration of glutathione.[24] Furthermore, hyperglycemia induced by corticotropin can be reduced by intravenous glutathione.[25] These observations prompted Butterfield[26] to administer the SH compound BAL (dimercaptopropanol) in two cases of insulin resistant diabetes with an apparent improvement of the glucose tolerance. The effect of BAL treatment in the present case of insulin resistant diabetes is in agreement with these observations.

SUMMARY

A patient with insulin resistant diabetes is described who required up to 1,500 units of insulin per day for

control of glycosuria and acetonuria.

No endocrinologic or metabolic disturbances were found that could explain the insulin resistance.

An insulin neutralizing factor in the serum of the patient was demonstrated by the Lowell mouse test and by the rat diaphragm test, measuring glucose uptake and glycogen synthesis.

The serum contained skin sensitizing antibodies and an antibody which agglutinated insulin-coated tannic acid treated red blood cells. Paper electrophoresis did not show increased gamma globulin values.

SUMMARIO IN INTERLINGUA

Diabete Insulino-Resistente con Anticorpores a Insulina: Reporto de un Caso

Es describite un patiente con diabete insulino-resistente, qui requireva usque a 1500 unitates de insulina per die pro le dominio de su glycosuria e acetonuria.

Esseva trovate nulle disturbationes endocrinologic o metabolic que haberea explicate le resistentia a insulina.

Un factor insulino-neutralisante esseva demonstrate in le sero del patiente per medio del test de Lowell a muses e del test a diaphragma de ratto, que mesura la acceptation de glucosa e le synthese de glycogeno.

Le sero contineva anticorpores cuti-sensibilisante e un anticorpore que agglutinava erythrocytos tractate con acido tannic e revestite de insulina. Electrophorese a papiro non monstrava augmentate valores de globulina gamma.

ACKNOWLEDGMENT

The authors wish to thank Prof. A. de Vries and Dr. M. Tur for valuable aid and criticism.

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