

A CASE OF COEXISTING INSULIN ALLERGY AND INSULIN RESISTANCE*

WILLIAM B. SHERMAN, M.D., NEW YORK, N. Y.

INSULIN resistance in diabetes is manifested by an insulin requirement considerably higher than that of a completely depancreatized man. Various writers have set arbitrary limits of 100 to 200 units of insulin daily as defining resistance. In the absence of known causative factors such as infection, acidosis, and liver disease, insulin resistance has been attributed to the presence of either an antagonistic hormone or an antibody reacting with insulin.

Crystalline insulin is a protein, and although of relatively low antigenicity in animals, it not infrequently causes allergic reactions, manifested by local or general urticaria, in human beings.^{1, 2} The coexistence of allergy and resistance to insulin in diabetic patients has been reported more frequently than might be attributed to chance.^{1, 3-11} The suggestion has been offered that the allergic reaction to insulin injected subcutaneously might delay its absorption,^{5, 11, 12} but in some cases of typical insulin resistance the hormone has been shown to be equally ineffective when injected intravenously.^{9, 13}

Lerman¹⁴ demonstrated the presence of a specific precipitin for crystalline insulin in the serum of one patient who was allergic and resistant to insulin, but failed to detect such a reaction in the sera of five other patients. Tuft¹⁵ also found a precipitin in the serum of a patient allergic but not resistant to insulin. Other observers have reported nonspecific or negative results with precipitin tests.⁹ Cannon and Marshall¹² with the collodion particle method were able to demonstrate antibodies for insulin in the sera of some patients, but neither they nor Lowell⁹ had consistently positive results.

Several investigators have attempted to demonstrate the presence of an insulin-neutralizing or diabetogenic factor in the sera of insulin-resistant patients by injecting the sera into rabbits or mice.^{8, 9, 13, 17-19} The most conclusive evidence of an insulin-neutralizing substance has been reported by Lowell in experiments on mice.^{9, 16} He also studied the difference in reaction to homologous and heterologous insulin by using insulin prepared from human pancreas as well as crystalline insulin from animal sources.

CASE REPORT

G. S., a 38-year-old housewife, was admitted to Presbyterian Hospital Oct. 18, 1948, because of diabetic acidosis. Mild diabetes had been first noted 5 years previously during pregnancy. Two years later, during an infection of her thumb, glycosuria had become marked and she was given protamine zinc insulin 10 units daily for one week. During this period she developed urticaria. No further insulin was given until 10 days before admission when

*From the Department of Medicine of the College of Physicians and Surgeons, Columbia University, The Presbyterian Hospital, New York, and the laboratories of the Department of Allergy, Roosevelt Hospital, New York.

protamine zinc insulin, 10 units daily, was again started because of a loss of 6 pounds in 3 months. Each injection of insulin produced a local urticarial reaction, and two days before admission generalized urticaria with periorbital edema developed. The insulin was discontinued and the urticaria controlled with Neo-Antergan. She became increasingly weak and drowsy and because the urine showed strong reactions for glucose and acetone she was admitted to the hospital. There was a past history of urticaria apparently due to a deodorant and a sister of the patient had hay fever.

On examination, the patient was slightly built, somnolent, dyspneic after talking, and mildly dehydrated. The odor of acetone was noted on her breath. The hemoglobin was 12.0 Gm. per cent, red blood count 3,500,000, white blood count 23,760, with polymorphonuclears 92 per cent, lymphocytes 5 per cent, and monocytes 3 per cent; the hematocrit was 40. The blood sugar was 394 mg. per 100 ml., the carbon dioxide combining power 28 vol. per cent. The urine showed 4 plus reactions for glucose, acetone, and diacetic acid.

During the first 8 hours the patient received infusions of glucose and saline with 120 units of standard insulin, but the reactions of the urine were not affected, the carbon dioxide combining power of the blood fell to 14 vol. per cent, the blood sugar was 388 mg. per 100 milliliter. At this time further infusions of dextrose and saline were given with the addition of sodium bicarbonate which restored the carbon dioxide combining power to 60 vol. per cent. Insulin was administered after each voided specimen was tested and during the second day 1,305 units were given. Because of an urticarial reaction at the site of each injection of standard insulin, crystalline insulin from beef and pork sources was substituted and Pyribenzamine, 50 mg., was given every 3 hours. This satisfactorily controlled the urticaria, and the general condition improved but the urine continued to give a 3 plus reaction for glucose.

The administration of insulin according to the reaction of the urine was continued, the total dosage on the third day was 1,030 units and on the fourth 1,675 units. Thereafter, the insulin dosage was gradually decreased although occasional specimens of urine continued to show glucose, 3 to 4 plus for the first 10 days. On the eleventh day the patient received a total of 230 units of crystalline insulin, had a mild hypoglycemic shock and showed no more than 1 plus glucose in any specimen of urine. During the following 3 weeks, various combinations of crystalline and protamine zinc insulin were given with total daily doses of 85 to 180 units but regulation was never satisfactory and urine specimens showing 3 or 4 plus glucose occurred almost every day, interspersed with occasional hypoglycemic reactions. The patient continued to have local swellings at the sites of injections of protamine zinc insulin, but no generalized urticaria. On November 20, globin insulin was substituted for protamine zinc, at first supplemented with crystalline insulin, then in daily doses of 130 to 145 units without supplements. She was discharged from the hospital December 3, with globin insulin 145 units daily and a diet of carbohydrate 100 Gm., protein 120 Gm., and fat 180 Gm. The urine at that time showed glucose 1 to 2 plus. The patient was then free from urticaria without Pyribenzamine.

Additional laboratory studies done to rule out known causes of insulin resistance showed serum bilirubin a faint trace, cephalin flocculation negative, thymol turbidity negative, and the basal metabolic rate plus 18 per cent. X-rays of the skull and chest were normal, as were visual fields.

On Jan. 24, 1949, she was seen in the outpatient clinic, the 24-hour urine gave negative reactions for glucose, on globin insulin 135 units daily.

Insulin Neutralization Studies.—Three specimens of serum, S-1, S-2, and S-3 obtained on the second, seventeenth, and thirtieth days of hospitalization were tested for insulin-neutralizing activity in mice by a method similar to that of Lowell.⁹ Five parts of each serum were mixed with one part of a solution of crystalline insulin, 40 units per ml., diluted 1:100 with saline. The glucose contents of the 3 sera were 600, 50, and 230 mg. per 100 ml., respectively. Because of the marked differences of glucose content, a separate mix-

ture of pooled normal serum and insulin was prepared as a control for each sample of the patient's serum. Sufficient 5 per cent glucose was added to each control mixture to make the total glucose content the same as that of the corresponding mixture made with the patient's serum, and equal volumes of saline were added to the mixtures containing diabetic serum. All mixtures were allowed to stand in the refrigerator for 24 hours.

White mice weighing approximately 8 to 9 Gm., from which food but not water had been withheld for 24 hours, were used. Five-tenths ml. of one of the serum-insulin mixtures was injected into the abdomen of each mouse. The mice were kept in an incubator at 35 to 37° C. and observed for 90 minutes for evidence of insulin shock which was usually manifested by convulsions, coma, general weakness, or paresis of the hind legs. A prompt injection of 0.5 ml. of 5 per cent glucose relieved the symptoms which occurred.

As shown in Table I, all of the control groups of mice reacted similarly, with 83 to 100 per cent showing symptoms of shock. Neither the added glucose (corresponding to 600 mg./100 ml. of serum in the first group) or the slight dilution of insulin it introduced caused appreciable differences. Two of 10 mice receiving the mixture prepared with serum S-1 developed symptoms of insulin shock, as did 3 of 11 mice treated with the mixture containing serum S-2, and 5 of 10 mice receiving the mixture of serum S-3. A similar mixture was prepared with serum S-1 diluted 1:10 with saline. All of the 5 mice injected with this mixture developed symptoms, as did the corresponding controls.

A portion of serum S-1 was heated 4 hours in a water bath at 56° C. and a similar mixture with insulin was prepared. Of 5 mice treated with this mixture, one developed mild shock, the proportion being the same as with the same serum unheated.

A mixture of 5 parts of serum S-2 and one part of human insulin 24 units (prepared by Dr. F. C. Lowell) diluted 1:60 with saline was made, also a corresponding control mixture of human insulin with pooled normal serum. Six mice were treated with each mixture and 5 in each group developed symptoms of insulin shock.

TABLE I. MOUSE PROTECTIVE TESTS

SERUM	GLUCOSE CONTENT MG./100 ML.	MICE USED	MICE SHOWING SHOCK SYMPTOMS	MICE SHOWING NO SYMPTOMS
<i>Crystalline Insulin</i>				
S-1	600	10	2	8
Control (glucose added)	600	11	10	1
S-1 (heated 4 hr. at 56° C.)	600	5	1	4
S-1 (diluted 1:10)	60	5	5	0
Control	60	5	5	0
S-2	50	11	3	8
Control	60	10	9	1
S-3	230	10	5	5
Control (glucose added)	230	10	9	1
<i>Human Insulin</i>				
S-2	50	6	5	1
Control	60	6	5	1

Passive Transfer Studies.—Sites were prepared in the skin of a normal test subject by the intracutaneous injection of 0.1 ml. of sera S-1, S-2, and S-3 undiluted and in dilutions of 1:10, 1:100, 1:1,000, and 1:10,000. These sites were tested 48 hours later with approximately 0.025 ml. each of crystalline (beef and pork) insulin 40 units per milliliter. As shown in Table II, serum S-1 transferred sensitivity in a dilution of 1:1,000, S-2 and S-3 in dilutions of 1:100.

Sites prepared with serum S-1 and tested with human insulin 24 units per ml. gave no reaction.

A sample of serum S-1 heated 4 hours at 56° C. failed to sensitize skin to crystalline insulin.

Blocking Antibody Study.—An attempt was made to detect in serum S-1 a thermostable antibody blocking the allergenic activity of crystalline insulin. Samples of serum S-1 and pooled normal serum were heated 4 hours at 56° C. and mixtures prepared each containing 0.1 ml. of heated serum, 0.1 ml. of successive ten-fold dilutions of crystalline insulin 40 units per ml. and 0.8 ml. of saline. After standing 24 hours in the refrigerator, each of these mixtures was tested on passive transfer sites sensitized with 0.1 ml. of unheated serum S-1. As shown in Table III, the results were equivocal.

Precipitin Tests.—Precipitin tests by the ring method were carried out with the 3 samples of serum and crystalline insulin, the reaction of the insulin being adjusted to pH 7.5 by addition of phosphate buffer. The reactions were negative with all 3 sera.

DISCUSSION

The patient showed evidence of allergy and marked resistance to insulin, both of which decreased while treatment with insulin was continued, Pyribenzamine being used to control the allergic manifestations. Sera drawn at intervals during the first month of treatment showed evidence of a factor neutralizing the hormone activity of heterologous crystalline (beef and pork) insulin, but not that of human insulin. These sera also contained a skin-sensitizing antibody reacting with crystalline insulin but not human insulin. The insulin-neutralizing factor differed from the skin-sensitizing antibody in that it was not affected by heating 4 hours at 56° C., which destroyed the sensitizing property. During the period of observation, both the neutralizing factor and the sensitizing antibody decreased, concomitant with decrease of clinical evidence of allergy and resistance.

The action of serum of the resistant patient in protecting mice against the effects of insulin was similar to that reported by Lowell.⁹ The amounts of serum and insulin injected were, likewise, similar to those used by Lowell, but the mice were considerably smaller, weighing 8 to 9 Gm. instead of 20 to 30 Grams. As a result, the incidence of shock in the control groups of mice was approximately 90 per cent as compared to 50 per cent in Lowell's series, but the protection afforded by the resistant sera was less complete.

The specificity of the insulin-neutralizing factor in reacting with beef and pork insulin but not with human insulin strongly suggests that the resistance in this case was due to an antibody combining with an inactivating heterologous insulin. The presence of the skin-sensitizing antibody offered further evidence

that the patient was reacting to insulin as an antigen. While it is probable that resistance in this case was due to an antibody, it appears likely in some other cases reported in the literature, insulin resistance was due to hormones rather than antibodies.

TABLE II. PASSIVE TRANSFER TESTS

<i>Sites Tested With Crystalline Insulin</i>				
SERUM	S-1	S-2	S-3	S-1 HEATED 4 HR. AT 56° C.
Undiluted	++++	++	+++	0
1:10	+++	++	++	0
1:100	++	+	+	0
1:1000	+	0	0	0
1:10,000	0			

Sites Tested With Human Insulin 24 Units

SERUM	S-1
Undiluted	0
1:10	0
1:100	0

TABLE III. BLOCKING ANTIBODY TEST

<i>Each Site Sensitized With 0.1 Ml. of Serum S-1</i>	
SITES TESTED AFTER 48 HR. WITH MIXTURES OF SERUM HEATED 4 HR. AT 56° C. AND CRYSTALLINE INSULIN	REACTION
1. Serum S-1 and insulin u 40	++++
1. Serum S-1 and insulin u 4	+++
3. Serum S-1 and insulin u 0.4	++
4. Serum S-1 and insulin u 0.04	±
5. Serum S-1 and insulin u 0.004	0
6. Control serum and insulin u 40	+++
7. Control serum and insulin u 4	+++
8. Control serum and insulin u 0.4	+++
9. Control serum and insulin u 0.04	+
10. Control serum and insulin u 0.004	0

The failure to demonstrate an antibody blocking the allergenic activity of heterologous insulin may well have been due to insufficient sensitivity of the test used. The mouse protective tests indicated that 0.1 ml. of serum S-1 neutralized the hormone activity of 0.008 units of insulin but not of 0.08 units. If the serum neutralized the allergenic activity of the same amount of insulin, its activity would not be clearly apparent in the blocking antibody test performed with the sensitizing serum available. The existence of such a blocking antibody for insulin was demonstrated by Loveless²⁰ in a serum, 1 ml. of which blocked the allergenic activity of 0.1 unit of crystalline insulin.

SUMMARY

1. The serum of a patient with coexisting allergy and resistance to crystalline insulin showed evidence of a factor protecting mice against heterologous insulin but not against human insulin. The specificity of this insulin-neutralizing factor suggested that it was an antibody.

2. Skin-sensitizing antibody reacting with heterologous but not human insulin was also demonstrated in the serum.

3. The insulin-neutralizing activity of the serum was not affected by heating four hours at 56° C., which destroyed the skin-sensitizing antibody.

4. Under the conditions of the experiment, it was not possible to demonstrate antibody blocking the allergenic effect of crystalline insulin.

5. Precipitin tests with the serum and crystalline insulin gave negative results.

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