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IMMUNOLOGIC STUDIES IN INSULIN RESISTANCE

I. REPORT OF A CASE EXHIBITING VARIATIONS IN RESISTANCE AND ALLERGY TO INSULIN

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Extreme resistance to insulin has been reported in a small number of diabetic patients who, for varying periods, have tolerated ten to fifty times as much insulin as that commonly required for the control of diabetes. Even with these very large doses, control of the diabetic state has not always been achieved. The subject of resistance to insulin has been reviewed recently (1). The high tolerance exhibited by these patients has been tentatively ascribed to lack of some factor necessary for the action of insulin, to endocrine imbalance or abnormality, to allergy, or to a neutralizing antibody. Previous reports contained evidence for the presence of a neutralizing factor in the blood of an insulin-resistant patient, which appeared to exhibit specificity and which was thought to be an antibody for insulin, distinct from the allergic antibody (2, 3). The course of this patient has now been followed for about 15 months and studies made during this period lend considerable support to the view that the resistance in this patient was immunologic in nature.

CASE REPORT

Patient A. M. is a 50-year-old white married female. At the age of 19, she underwent a pelvic operation following a pregnancy after which there was complete cessation of menstruation. She had been well otherwise until 1931 when she developed an appendiceal abscess which was drained surgically at the Boston City Hospital. During this admission, a diagnosis of diabetes mellitus was made and treatment with insulin was begun. No adverse reactions occurred and the patient stated that her urine became free of sugar. On discharge, she was advised to follow a diet and to take no insulin. In 1939, she was seen in the Out-Patient Department of the Boston City Hospital and treatment with protamine zinc insulin (Lilly) was begun because of glycosuria and acetonuria. About one week after resuming treatment, there were marked local reactions to the injected insulin and, at times, there were also generalized urticaria and a constricting feeling in the chest. Intracutaneous tests with beef, pork, and lamb

as well as crystalline insulins indicated allergy to all these and the injections were discontinued (4).

In August 1941, she was admitted to the Boston City Hospital complaining of aching and paresthesias of both legs and feet of 2 months' duration. There had been also loss of appetite, episodes of nausea and vomiting, weakness, and weight loss. Physical examination revealed obesity, muscle tenderness of the legs, and absent knee and ankle jerks. The blood pressure was 140/80. Laboratory studies showed a three plus reaction for sugar and acetone in the urine. Albumin and many white cells were also present. Culture of the urine yielded *E. coli*. The blood hemoglobin was 78 per cent and the white count was 8,400. The differential count showed no abnormality and there was no eosinophilia. Other findings were as follows: Hinton negative; NPN 34; FBS 215; CO₂ combining power 38 per cent; chlorides 101 m.eq. A diagnosis of diabetic acidosis was made and the patient was given infusions of saline. Sulfadiazine was given by mouth for the urinary infection.

The patient warned the house officer that she was allergic to insulin. A dose of 4 units (0.1 ml. of U 40) of regular insulin was given intracutaneously on August 5, 1941. This was quickly followed by severe generalized urticaria, difficulty in breathing, and a fall in blood pressure with loss of consciousness. Adrenalin given intravenously and intramuscularly gave relief. Two days later desensitization was begun with an initial dose of 0.001 unit of crystalline insulin, subcutaneously. After 3 days, during which increasing amounts of insulin were given, 8 units were well tolerated in a single dose. Thereafter, larger doses were given until 11 days after the first dose of insulin she could tolerate 300 units in a day and 2 days later she received 570 units in a day. These amounts of insulin were given without having any observable effect on the diabetic state. No fall in the blood sugar followed the intravenous injection of 30 units of crystalline insulin. Finally, on September 2, 1941, 26 days after desensitization was begun, 860 units of regular insulin were given slowly by continuous intravenous infusion, over a period of 6 hours. Blood sugar determinations during and after this period showed no fall, but on the contrary, a gradual rise from 250 mgm. per 100 ml. to 364 mgm. per 100 ml. The injection was finally stopped because of severe urticaria, nausea, and vomiting. The total dose of insulin given during this admission was approximately 2,500 units. No further attempts were made to treat the patient with insulin at this time. X-rays of the skull showed no ab-

normalities of the sella turcica. The patient was discharged on the 33rd hospital day with instructions to follow a diet and to take no insulin.

During the following 4 months, she had fairly frequent headaches, some aching and burning of the legs and feet, nocturia one to three times, and considerable drowsiness.

In February 1942, she was admitted to the Evans Memorial Hospital for further study. The findings on physical examination were the same as those noted above with the addition of diabetic retinitis. There was no ketonuria, but the urine constantly contained large amounts of sugar and there were many white cells and variable amounts of albumin. Culture of the urine yielded *E. coli*. X-rays of the skull were negative. Endermal injection of 0.05 ml. of U 40 crystalline insulin diluted 1:100 (0.02 units) produced local whealing, erythema, and itching, associated with transitory mild generalized itching. Desensitization was begun on February 18, 1942 with increasing doses of insulin. Several attacks of generalized urticaria and constriction in the chest prevented rapid increases in the dosage. No insulin effect was noted until the third day (February 20) when she complained of dizziness after receiving 102 units, subcutaneously, over a period of 8 hours. The urine became free of sugar and the blood sugar fell to 84 mgm. per 100 ml. During the next 2 days, the patient received 24 and 44 units, respectively. These amounts were sufficient to clear the urine of sugar but the fasting blood sugar remained elevated. No systemic allergic reactions occurred, and redness, swelling, and itching at the site of the injections of insulin were absent or minimal. At this time, it appeared that the patient had lost her resistance to insulin and no further difficulty in the control of the diabetes was anticipated.

During the following 5 days, various tests were done with insulin and because of these, control of the diabetes was not attempted. When insulin therapy was again started for control of the diabetes, it was found that the patient had become resistant. On the eleventh and twelfth days after desensitization was begun, a total of 458 units of insulin was given subcutaneously without having any apparent effect on the diabetic state. Thus it appeared that the patient had again become resistant. Systemic manifestations of allergy did not occur, but local itching and redness were marked.

On March 10, 21 days after desensitization was started, 30 units of crystalline insulin were given intravenously without causing a fall in blood sugar. In a similar test with human insulin on March 11, the blood sugar fell from 360 mgm. per 100 ml. to 84 mgm. per 100 ml. in 90 minutes, indicating that the patient was not resistant to this insulin. These tests have been reported previously (2). No allergic reaction followed the injection of crystalline insulin but the test with human insulin caused severe urticaria, constriction in the chest, and nausea and vomiting. Treatment with insulin was then abandoned, the patient having received a total of approximately 1,500 units in 23 days. Sulfathiazole was given for the urinary infection and thiamin chloride for the peripheral neuritis.

She was discharged on the 36th hospital day with instructions to follow a diet.

She remained well until May 1942 when she developed scarlet fever for which she was hospitalized. During this period, the fasting blood sugar was consistently high and the urine was strongly positive for sugar. She made an uneventful recovery and received no insulin during the illness. For the following 4 months, she felt fairly well but developed increasingly severe symptoms of peripheral neuritis during the 5th month.

She was again admitted to the Evans Memorial Hospital in October 1942 having had no insulin for 8 months. The physical findings were unchanged. Intracutaneous tests with beef, pork, and crystalline insulin, and with a preparation of human insulin to be described below, were strongly positive. Control of the diabetes was again attempted and the sequence of events was very similar to that of the admission in the spring of 1942. The patient's course for the first 12 days of insulin therapy is shown in Figure 1. The high degree of allergy again prevented the administration of effective amounts of insulin during the first day. An attempt to desensitize the patient by giving daily injections of 10 units in divided doses during the first 3 days was unsuccessful. Fifty-six units given in the course of 6 hours on the fourth day caused severe urticaria and tightness in the chest. On the fifth day, November 3, 1942, 140 units given subcutaneously over a period of 8 hours were tolerated. This amount caused a hypoglycemic reaction, the blood sugar falling to 82 mgm. per 100 ml. A second reaction occurred when 30 units more were given in divided doses. Thus, for the second time, relatively small amounts of crystalline insulin were effective. On the sixth day (November 4), the fasting blood sugar was 98 mgm. per 100 ml., and only 46 units of insulin were required to prevent the excretion of sugar in the urine. It should be noted, however, that the small amount of insulin required may have been due in part to the low caloric intake on that day. A temporary decrease in the intensity of the local reactions at the site of the injections was again noted.

From the seventh to the tenth day, the daily dose of insulin was steadily increased. The fasting blood sugars remained elevated and some sugar was excreted in the urine. This suggested that resistance to insulin was returning. On the eleventh day (November 9), after 200 units had been given subcutaneously and the urine continued to show a strong reduction, 82 units were given intravenously in divided doses over a period of 3 hours. This caused a hypoglycemic reaction, the blood sugar fell to 67 mgm. per 100 ml. and the urine became free of sugar. On the twelfth day, 20 units given subcutaneously followed by the intravenous injection of 140 units given over a period of 7 hours caused a decrease in the amount of sugar in the urine temporarily but no hypoglycemic reaction occurred. No insulin was given on the thirteenth day and the total excretion of glucose rose to 83 grams, indicating that the increasing daily doses of insulin from the seventh to the twelfth day were having some effect on the diabetes.

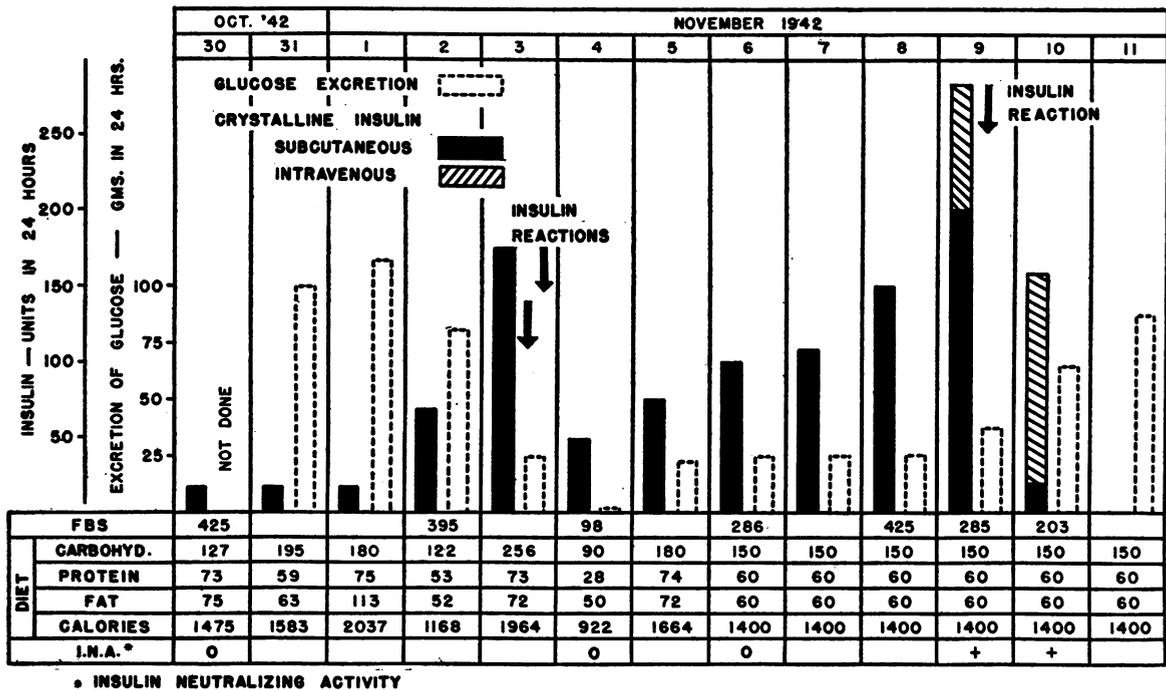


FIG. 1. RESPONSE TO TREATMENT AFTER 7 MONTHS WITHOUT INSULIN

On the 15th day (November 13) after desensitization was begun, an intravenous insulin tolerance test with 30 units of crystalline insulin showed a rise followed by a slight fall in the blood sugar (Figure 2). The patient was then desensitized with a preparation of human insulin and, on the following day, an intravenous insulin tolerance test with human insulin was done.

The human insulin used in this test was prepared during the summer of 1942. Assay was made by comparing its capacity to lower the blood sugar in rabbits with that of a known sample of commercial (Lilly) insulin. The glucose determinations were made in the fasting state and then at 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, and 5 hours after the injection of the insulin. Tests were done at weekly intervals. Four animals were used and each animal was tested 3 times with both insulins. The difficulty of making an accurate assay of insulin is well known and a definite strength cannot be ascribed to this sample of human insulin on the basis of the small number of tests done. However, all the tests indicated that the sample contained less than 30 U per ml. and it may have contained as little as 20 U per ml. On the basis of the tests done, a potency of 25 U per ml. was assigned to it but this must be regarded as only approximate. The intravenous injection of 0.6 ml. of this preparation in a fasting non-diabetic individual was followed by a fall in blood sugar from 106 mgm. per 100 ml. to 78 mgm. per 100 ml. in 45 minutes, with a subsequent rise to the fasting level, 90 minutes after the test was started. This fall in

blood sugar is not large and suggests that the preparation contained less than 25 units per ml.

A severe generalized allergic reaction followed the intravenous injection of human insulin in this patient on a previous occasion (2). Therefore, later in the day (November 13) on which the insulin tolerance test with 30 units of crystalline insulin was done, the patient was given graded doses of human insulin for desensitization. Injections were first made endermally, then subcutaneously, and finally intravenously. A total of approximately 50 units (2 ml.) of the preparation of human insulin was given in a period of about 6 hours. One hour after the last dose, the patient became weak and shaky. Orange juice gave relief. On the following day (November 14), the fasting blood sugar was 282 mgm. per 100 ml. The injection of 25 units (1.0 ml.) of human insulin intravenously was followed by a fall in the blood sugar to 122 mgm. per 100 ml. in 2 hours. There was no allergic reaction during this test. The results of the tests with crystalline and human insulins are shown in Figure 2.

Finally, on November 15, 1942, 60 units of regular pork insulin (Lilly) were given intravenously over a period of 35 minutes. The blood sugar was 310 mgm. per 100 ml. before the injections and at the end of 90 minutes was 278 mgm. per 100 ml. During this hospital admission, the patient received approximately 1500 units of insulin. She was discharged on the 17th hospital day with instructions to follow a diet and to take no insulin. Diagnoses: Diabetes mellitus; diabetic retinitis; resistance to insulin;

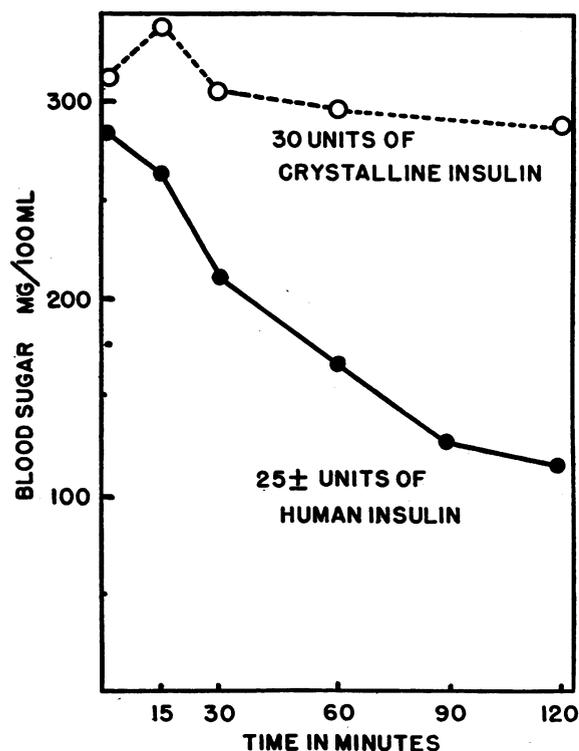


FIG. 2. INTRAVENOUS INSULIN TOLERANCE TESTS

allergy to insulin; peripheral neuritis; chronic pyelonephritis, *E. coli*; obesity.

DISCUSSION

This patient differs from the reported cases of insulin resistance in that she received relatively small amounts of insulin and there were long periods during which no treatment was given. Continuous administration of the huge doses of insulin usually required for resistant patients was not attempted because of the marked local and systemic allergic reactions and because of the poor prospect of benefit to the patient. Withholding of insulin was possible because a fair state of health could be maintained on diet alone.

There were 3 periods during which the patient received insulin and exhibited resistance. On 2 of these occasions, resistance was preceded by a very brief state of relative responsiveness to insulin which, in the first instance (February 20, 1942), was demonstrated on the third day, and in the second (November 3, 1942), on the fifth day after desensitization with insulin was begun.

The greater length of time required in the second instance was due to the mistake in believing that 10 units given daily would effect desensitization. No test for determining the responsiveness of the patient could be done until preliminary desensitization had been carried out. In each case, the temporary state of susceptibility to insulin was preceded by a period of 5 months or more during which the patient received no insulin whatsoever. It appears probable, therefore, that during the extended periods without treatment the resistance became less or disappeared. Resistance reappeared within about 10 to 12 days of beginning desensitization and persisted as long as insulin was given. Temporary responsiveness was not observed during the admission to the Boston City Hospital in the fall of 1941. This may have been due to inadequate dosage of insulin within the first 7 to 10 days of beginning desensitization.

The patient's course during the period of study is illustrated in Figure 3. This shows the relationship between the administration and withholding of insulin and the changes observed in the patient's resistance. The results of tests for insulin-neutralizing activity (I.N.A.) in the patient's serum are also indicated. These tests were carried out in mice and are described in detail in the second part of this study (5). A plus sign indicates the presence and a zero the absence of insulin-neutralizing activity in the serum. When the patient was demonstrably resistant to insulin, the blood showed insulin-neutralizing activity but blood obtained when the patient was responsive to insulin, or shortly beforehand, showed none. Neutralizing activity was apparently present in bloods obtained as long as 10 weeks after the administration of insulin had been stopped.

YEAR	1941					1942													
	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11			
RESISTANCE	+						0	+									0	+	
I.N.A.	+		+			0	0	+	+				0				0	0	+
INSULIN	GIVEN							WITHHELD					WITHHELD						GIVEN

FIG. 3. CHANGES IN THE DEGREE OF INSULIN RESISTANCE AND OF INSULIN NEUTRALIZING ACTIVITY (I.N.A.) OF THE SERUM IN RELATION TO THE ADMINISTRATION OF INSULIN

These observations are consistent with the view that resistance to insulin in this patient was immunologic in nature and that the insulin-neutralizing activity of the patient's serum was due to the presence of a neutralizing antibody for insulin. This is also supported by the patient's greater responsiveness to human than to crystalline insulin, indicating some degree of specificity in the resistance. Tests in mice also indicated a similar specificity (2, 5). Immunologic identity for insulins derived from a number of mammalian species including man has been claimed (6). This conclusion was reached on the basis of cross reactions in complement fixation tests and anaphylaxis in guinea pigs. However, it is commonly agreed that cross reactions in immunologic systems, done without quantitative control, indicate similarity but do not prove identity.

Resistance to insulin may not always be demonstrably specific. A second patient, C. S.,¹ was found to be resistant to both human and commercial insulins. This was a 60-year-old white female who also had lipodystrophy. She had required daily doses of insulin ranging from 500 to 2,500 units for a period of 9 months. Injection of the large doses of insulin required for control of the diabetes usually caused some local redness and when extremely large doses were given, a few hives occasionally appeared. A single dose of approximately 50 units (2 ml.) of the same preparation of human insulin given the first patient, A. M., was injected intravenously in the fasting state. This caused no discomfort but 3 hives appeared on the thighs and arm. The blood sugar was 274 mgm. per 100 ml. before the injection and fell gradually to 247 mgm. per 100 ml. at the end of 2 hours. This fall was not considered significant. The blood of this patient also showed insulin-neutralizing activity when tested with crystalline (beef and pork) insulin, and this was also demonstrable in tests with human insulin.

One explanation for the difference in the responses of the 2 insulin resistant patients to human insulin was the development of a less specific antibody in the second patient than in

the first. An analogy is seen in experiments by Hooker and Boyd (7, 8) which were carried out in rabbits. For example, early in the course of injections of chicken ovalbumin, antibody of a high degree of specificity was produced. When the injections of antigen were continued and the animals became "hyper-immune," the antibody then precipitated duck ovalbumin, a related antigen. To return to the 2 insulin resistant patients, the first, A. M., received approximately 5,000 units of insulin over a period of about 14 months, whereas the second received an estimated total of about 200,000 units in 9 months, 40 times the first figure. Thus, compared to the first patient, the second might be considered to be "hyper-immune" with respect to insulin.

Marked allergy to insulin as manifested by a tendency to generalized urticaria and constriction in the chest was not observed to be associated with resistance, and the conclusion seems warranted that the two were distinct and possibly independent immunologic mechanisms. For example, on 2 occasions in the clinical course of A. M. (February and October 1942), a high degree of allergy was observed only a few days before responsiveness to insulin was demonstrated. This suggests that the allergic state occurred in the absence of resistance. The objection may be raised that a test for susceptibility to insulin was never carried out *until* desensitization was accomplished and it may be argued that desensitization itself was the cause of the reduced resistance. The rôle of desensitization in inducing responsiveness may be doubted, however, because resistance was observed while the patient was still in the desensitized state. If resistance and the state of desensitization could coexist, it seems unlikely that the latter would also bring about the disappearance of the former. The failure to demonstrate the insulin-neutralizing factor in the blood before desensitization was begun, is added evidence for the view that allergy could exist in this patient in the absence of resistance. If this is correct, the development of resistance in A. M. must be attributed to something other than allergy to insulin.

A theory which explains many of the features exhibited by A. M. is given below. Two distinct antibodies for insulin are postulated (2, 5, 10).

¹Opportunity to study this patient in the Deaconess Hospital, Boston, was kindly afforded by Dr. Howard F. Root.

One of these is the insulin-neutralizing factor which is considered to be analogous to, but not necessarily identical with, antibody produced in animals following the injection of a protein. Production of antibody of this type may be induced by injections of antigen and when the injections are stopped, the titer falls slowly. The second antibody is the allergic antibody, also referred to as atopic reagin and is only demonstrable in the skin of suitable recipients (Prausnitz-Küstner reaction). An important peculiarity of this antibody is that once it makes its appearance in the blood, it may persist in undiminished titer for long periods without antigenic stimulus (9).

The proposed theory is represented schematically in Figure 4. A complete cycle is shown

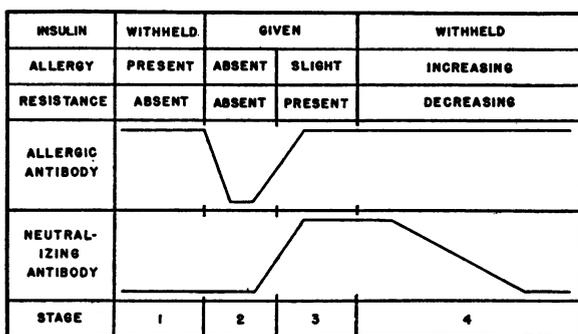


FIG. 4. SCHEMATIC REPRESENTATION OF THE SUGGESTED RELATIONSHIP BETWEEN THE ALLERGIC AND THE RESISTANT STATES IN A. M.

illustrating 4 consecutive stages: (1) a high degree of allergy; (2) desensitization with responsiveness; (3) desensitization with resistance; (4) the return of the allergic state and the loss of resistance. The period from January to October 1942 (Figure 3) represents such a cycle. Before desensitization, the allergic antibody is present and the neutralizing factor is absent (stage I, Figure 4). Allergic manifestations are easily induced and it is assumed that resistance is not present at this time. With increasing doses of insulin, neutralization of the allergic antibody takes place in the patient. The titer falls and desensitization is accomplished. The neutralizing antibody has not made its appearance and relatively small doses of insulin can induce hypoglycemic reactions. This may be called the first phase of desensitization. The

second phase (stage 3) is characterized by the appearance of the insulin-neutralizing factor as a result of the antigenic stimulus. There are 2 consequences: (1) reappearance of resistance; (2) a return of the allergic antibody, but without the development of systemic manifestations of allergy. This is explained by the greater avidity (10) of the neutralizing antibody for the antigen as compared with the allergic antibody, with the result that the former combines preferentially with insulin. It is assumed that this affords protection by preventing un-neutralized insulin from reaching the sensitized cells of the body. Systemic manifestations of allergy can now be produced only if large amounts of insulin are given (see above, hospital admission of August 1941). When the administration of insulin is stopped and no further antigenic stimulus occurs (stage 4), the neutralizing factor slowly disappears. The allergic antibody persists, however, and the patient finally returns to the allergic state and may again exhibit responsiveness to insulin after desensitization. The cycle is now complete.

This theory can also explain the marked systemic allergic reaction which occurred in A. M. in the first tolerance test with human insulin (2). This was done at a time when she was desensitized and resistant to *crystalline* insulin. After injection of the human preparation, generalized urticaria occurred which was followed by a hypoglycemic reaction, another example of the presence of allergy in the absence of resistance to insulin. Neutralizing activity for crystalline but not for human insulin was demonstrable in serum taken at that time (2). However, sensitization of normal skin to both human and crystalline insulin was easily accomplished. The allergic reaction as well as the lack of resistance may be ascribed to the absence of a neutralizing antibody for human insulin with the result that neutralization did not occur, and insulin remained free to produce allergic symptoms. If this explanation is correct, one may also infer that the allergic antibody was less specific than the neutralizing factor.

SUMMARY AND CONCLUSIONS

A case of resistance to insulin, associated with a high degree of allergy to insulin, is reported.

Changes occurred in the degree of resistance which were apparently related to the giving and withholding of insulin. The resistance was specific in that human insulin caused a markedly greater fall in the blood sugar than did crystalline insulin. The findings in this patient support the view that the resistance to insulin is immunologic in nature.

The clinical course and the experimental findings indicate that allergy to insulin and resistance to insulin varied independently of each other. Two phases of desensitization were found, the first characterized by the absence, the second by the presence of resistance to insulin. It is suggested that the clinical observations may be explained by postulating 2 immune systems: (1) the allergic mechanism associated with the skin-sensitizing antibody, responsible for generalized urticaria, constriction in the chest, and collapse; (2) the insulin neutralizing mechanism associated with a neutralizing antibody for insulin and responsible for the patient's resistance to insulin.

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