THE ROLE OF NONPRECIPITATING INSULIN ANTIBODIES IN DIABETES [1]

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Interest in antibodies to insulin developed with the genesis of the first reaction at the site of insulin injection (1–3). It was almost inevitable that once the existence of antibodies to insulin had been suggested, the effect of these on the insulin itself would be questioned. Studies performed on the serum of insulin resistant patients provided further impetus to the concept that antibodies to insulin inhibit the action of insulin, thus necessitating the use of of higher doses than would otherwise have been the case (1, 4–17). In some instances the methods then available could demonstrate antibodies to insulin by direct skin testing, by passive transfer, by complement fixation or by the use of collodion particles (2, 3, 18, 19). It was learned that skin sensitizing antibodies were present only in patients who had allergic reactions to insulin and were independent of the patient's insulin requirements (3–7, 18, 20). This appeared to indicate that if an antibody were present in the insulin resistant patient, this antibody was distinct from the skin sensitizing antibody. In most cases, this suspected antibody did not precipitate insulin, fix complement, hemolyze red cells or do anything else easily recognized. Therefore, the presence of this antibody had to be determined by neutralization or reduction of the physiological action of insulin.

The following experimental approaches have been used:

1. Treatment of insulin resistant patients with human insulin (6, 20).
2. Studies of the effect of the plasma of selected patients or animals on the uptake of glucose by a rat diaphragm, with and without added insulin (10, 15, 21–25).
3. Studies of the effect of selected plasma alone or with added insulin on animals which were also receiving an otherwise lethal or convulsive dose of insulin (5, 14, 15, 17, 20).
4. Studies of the effect of the plasma of selected patients on the degradation of insulin by rat slices and liver homogenates (21, 26).
5. Studies of the protective effect of gamma globulin from insulin resistant patients on animals receiving insulin (11–15).
6. Studies of the effect of the plasma of insulin resistant patients on an animal's blood sugar (8, 9, 13, 15, 16, 20, 27).
7. Studies of the effect of selected plasma and added insulin on the blood sugar of animals (8, 9, 11, 12, 16, 27).

As a result of these various experiments, it is apparent that the inhibition of insulin may occur through several different mechanisms, all of which are by no means clearly understood. These mechanisms are of three general types: a) cellular, *i.e.*, by competition with or antagonism of the cellular function of insulin; b) enzymatic destruction of insulin; and c) binding of insulin to circulating protein.

Since an enzyme is a protein which has a definite affinity for its substrate, insulinase might be considered an antibody if it occurred as a response to the injection of insulin. However, this response has never been demonstrated clearly and indeed insulinase activity has been found in sera of patients and animals that have never received insulin (27, 28). It is the third type of inhibition which is most clearly due to an antibody and is the subject of this study. The binding of insulin by serum has been demonstrated only in patients and animals that have received injections of heterogenous insulin. Indeed, it is a regular response to such injections (29, 30).

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MATERIALS AND METHODS

In the preceding paper (31), a method was presented for demonstrating the binding of insulin by the gamma globulins in any given serum. Using this method the insulin binding by the serum of 48 patients was determined. The term "insulin binding" is used to designate the percentage of insulin which is bound by the gamma globulins of an individual serum under standardized conditions (31). Each serum was analyzed within 48 hours after it was obtained and then frozen at $-20°$ C. Since the concentrations of insulin in different lots of I^{131} insulin may vary somewhat, all of the results presented here were obtained from a simultaneous reanalysis of all the sera. The final and initial analyses did not differ in any case by more than 10 per cent. If less than 1 per cent of the radioactive insulin was bound by a 1:25 dilution of the individual serum, then this serum was classified in this paper as negative. Otherwise the actual per cent of insulin bound is given. Since the percentage binding is a result of both the amount of antibody present and the equilibrium constant of the antigen-antibody reaction, the results should not be interpreted as indication of the absolute amount of antibody present. However, due to the relatively slow turnover of globulins in humans, any sudden change in binding should reflect a similar change in the concentration of antibody. The amount of I^{131} insulin added under the conditions of the test is equivalent to 66 units per liter of undiluted serum. Since the amount of unlabeled insulin contained in the serum will, under almost any conceivable condition, be small in comparison to the labeled insulin added, and since the

TABLE I

Insulin binding of sera from insulin treated patients

Patient	Age	Sex	Insulin binding *per cent*	Insulin treatment *duration*	Daily dose of insulin in units			Comment
					Highest	Lowest	Most recent	
K. J.	43	F	0	16 days	20	5	5	No insulin for 2 mos. at time of study
R. H.	65	F	0	3 days	15	10	10	
			0	14 days				
			0	18 days				
L. R.	64	F	0	6 yrs.; then none for 6 yrs.			15	Serum studied 9 days after insulin was resumed.
A. M.	67	F	2	6 yrs.+	125	30	45–55	Forty-five years of diabetes.
R. A.	66	M	2	9 yrs.	60	30	40–45	Steroids 5 mos. Insulin requirements increasing.
A. G.	27	F	2	6 yrs.	125	50	90–100	
A. G.	64	M	3	6 mos.	20	20	20	
H. B.	43	M	3	1 mo.; discontinued for 1½ yrs.	50	20	30–45	Serum studied 11 days after resumption. Insulin requirements increasing.
P. S.	46	M	6	5 mos.	30	0	15–20	Third sample 4 mos. later showed titers rising.
			4	5 wks.				
J. B.	55	M	8	7 yrs.	30	15	15–30	Three sera. Requirements decreasing. Fasting and nonfasting determinations show equal titers.
G. S.	35	F	12	8 yrs.	80	25	25–35	Titer going up although insulin requirements decreasing.
			18					
			22					
F. D.	56	F	8	4 yrs.; then none for 4 days; then 2 days' treatment	275	?	35–45	Insulin requirements decreasing. Fasting and nonfasting determinations show equal titers.
H. R.	32	M	16	14 yrs.	45	25	30–45	
O. J.	53	M	17	7 yrs.	15	10	10–15	Irregular dosage.
M. G.	24	M	17	17 yrs.	?	?	35–40	Juvenile diabetic. Dosage decreasing. Patient uremic at time of study.
F. R.	50	F	21	6 mos.	40	26	30–40	
J. A.	17	F	24	4 yrs.	91	25	25–40	Juvenile diabetic. Requirements decreasing.
H. M.	65	F	26	3 yrs.	?	?	13–15	Fasting and nonfasting titers are equal.
S. S.	66	M	28	3½ yrs.	120	12	90–120	Requirements increasing.
F. H.	45	F	36	13 yrs.	600	30	60–80	Lawrence-Ziegler syndrome. Requirements decreasing.
L. R.	52	M	39	12 yrs.	80	10	60–80	Decreasing requirements. Many insulin reactions.
H. K.	48	M	47	30 yrs.	?	?	50–65	Juvenile diabetic. Requirements decreasing.
P. G.	75	M	56	? 10 yrs.	50	30	40–50	Hemochromatosis and hepatoma.
E. P.	53	F	See Figure 1					
S. I.	58	F	See Figure 2					

TABLE II

Insulin binding of sera from insulin treated patients whose insulin had been discontinued

Patient	Age	Sex	Insulin binding *per cent*	Insulin treatment *duration*	Daily dose of insulin in units			Comment
					Highest	Lowest	Most recent	
W. T.	56	M	0	Less than 2 mos.	10	5	0	No insulin for 2 mos. at time of study.
W. S.	72	M	0	4 mos.	10	5	0	No insulin for 1 yr. at start of study. Four samples over 6 mos. period although patient developed fever, furuncles and hyperglycemia.
E. B.	60	M	0	2–3 yrs.	?	?	0	No insulin for 1½ yrs. at time of first study. Eight mos. later serum still negative.
L. M.	56	F	0	3 yrs.	?	?	0	No insulin for 10 mos. at time of study. Patient's blood sugar was out of control with infection and fever at this time.
J. K.	44	M	3	7 mos.	30	0	0	Lente insulin. None for 2 wks.
R. A.	50	F	3	4 yrs.	20	0	0	No insulin for 3 yrs. Three subsequent sera show the same results.
R. C.	57	F	6 (8/31) 4 (11/20) 3 (12/4) 6 (3/30)	7 yrs.	30	0	20–30 0 0 5–30	Determinations on 8/31, 11/20, 12/4 and 3/30. Decreasing insulin requirements with insulin stopped on 9/21 and resumed on 3/24.
R. K.	59	F	7	13 yrs.	20	0	0	Insulin stopped 7 wks. prior to first determination; repeated 2½ mos. later and still 2+.
I. T.	56	F	8	3 yrs.	40	0	0	No insulin for 3 years.
E. C.	60	F	18 10 9	3 yrs.	20	5		First serum studied after 23 days of insulin following 8 mos. of no insulin. Second serum was studied 2½ mos. after insulin was again discontinued. Third serum studied 3½ mos. later when patient still not on insulin.

labeled and unlabeled insulin exchange freely (29), it seems unlikely that the prior injection of unlabeled insulin will have an appreciable effect on the result of the test.

RESULTS

Table I shows the relative insulin binding by the sera of 25 diabetic patients currently receiving insulin. The insulin dosage, duration of treatment and other pertinent clinical information are presented in abbreviated form. No clear relationship is apparent between the dosage of insulin or duration of treatment and the insulin binding of the patient's serum. However, 22 out of the 25 sera were positive. Two of the negative sera had received insulin for 18 days or less. One had received insulin for only 5 days following 6 years without treatment. One patient's serum began to bind insulin as early as 5 weeks after the onset of treatment.

Table II presents similar data for patients whose insulin has been discontinued, with or without reinstitution of insulin. It may be seen that in some patients the antibodies persist as long as three years after treatment has been stopped. However, the average insulin binding of the sera in this group is lower than in the group currently receiving insulin (Table I).

In two patients (P. S. and G. S.), a rise in insulin binding with duration of insulin treatment was observed despite a reduction in the insulin dosage. No differences could be demonstrated at different times of the day although blood sugar levels (and presumably exogenous insulin concentration) vary.

No evidence was obtained of a nonspecific anamnestic rise in insulin binding as a response to fever or infection, as suggested by Steigerwald and Spielmann (32). In other respects, however, these findings are consistent with both the time required and the individual variation observed in many other antigen-antibody systems.

Figure 1 shows the nonfasting and some fasting blood sugars, the daily insulin dosage and the insulin binding capacity of the sera from a 57 year

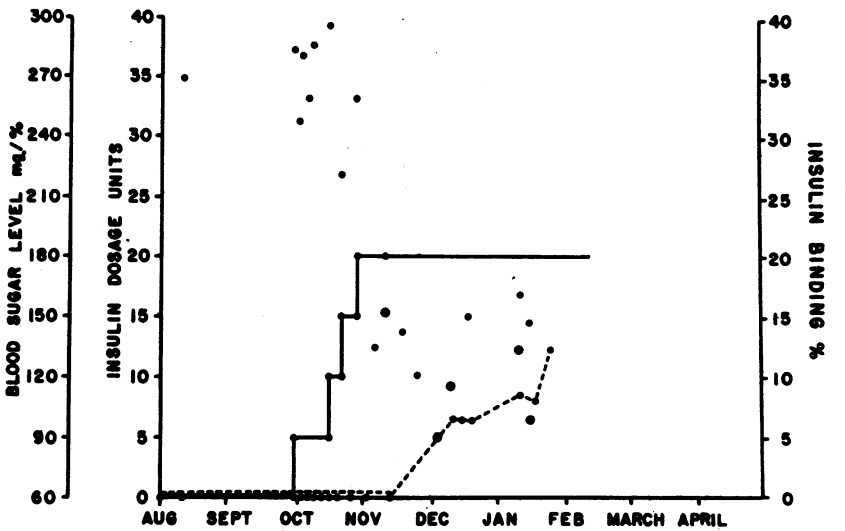

FIG. 1. INSULIN DOSAGE AND INSULIN BINDING OF THE SERA OF A DIABETIC PATIENT ON WHOM OBSERVATIONS WERE MADE FOR TWO MONTHS BEFORE BEGINNING OF THERAPY

The unbroken line represents insulin dosage, the broken line insulin binding, the isolated points nonfasting blood sugar, and the circles fasting blood sugar.

old Negro female who refused insulin for several months after diabetes was discovered. The time of development of insulin binding and its increase in titer in the face of reduced blood sugar are clearly demonstrated.

Figure 2 shows the fluctuations of the blood sugar of a 67 year old white female. The decrease in her insulin binding at a time when she was critically ill is demonstrated, as is the subsequent rise despite reduced insulin requirement and dosage.

Table III presents the negative controls studied. All are individuals who had never received insulin. These include one patient with hemochromatosis,

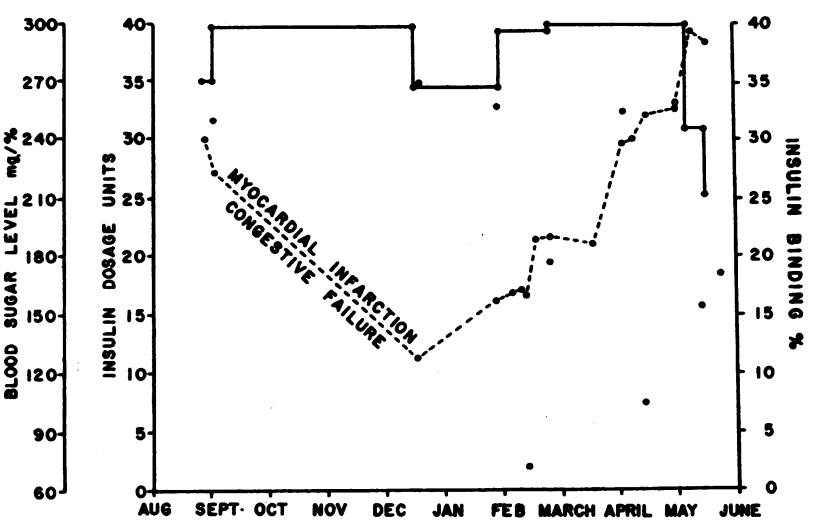

FIG. 2. THE INSULIN DOSAGE AND INSULIN BINDING OF THE SERA OF A DIABETIC PATIENT WHO WAS OBSERVED DURING A PERIOD OF CRITICAL ILLNESS

The unbroken line represents insulin dosage, the broken line insulin binding, and the isolated points nonfasting blood sugar.

one with a functioning islet-cell carcinoma with metastasis, two healthy subjects and several patients with various diseases. Two of these patients had very low serum gamma globulin values and a few had elevated total globulins. None of them demonstrated any insulin binding.

TABLE III

Twelve patients who have never received insulin, all without demonstrable insulin binding

Patient	Age	Sex	Comment
F. S.	60	M	Hemochromatosis, diabetes.
F. T.	61	M	Arteriosclerosis, diabetes.
S. W.	61	F	Functional islet cell adenocarcinoma with metastases.
B. W.	45	M	Blood dyscrasia and acquired hypogammaglobulinemia.
T. H.	50	M	Hypogammaglobulinemia.
F. H.	52	M	Normal male.
A. A.	55	M	Asthmatic bronchitis.
P. E.	62	M	Cerebral arteriosclerosis.
H. L.	56	M	Gouty nephritis, hypertension, congestive failure.
W. C.	21	M	Normal male.
M. P.	30	F	Allergic asthma.
M. S.	52	F	Myxedema.

DISCUSSION

The findings of the present work may be summarized as follows:

1. No insulin binding was found in patients, diabetic or otherwise, who had not received insulin.

2. Insulin binding was present in all of 23 patients who had received insulin recently for more than one month.

3. Antibodies were demonstrable in two patients who had received no insulin for three years.

4. An increase in insulin binding of a patient's serum was never found to precede or immediately follow an increase in insulin requirement or dosage.

5. No consistent relationship could be demonstrated between the insulin binding and the insulin requirements of patients receiving insulin.

On the basis of the evidence presented in this and the preceding paper, it would appear that the insulin binding demonstrated by precipitation with anti-human gamma globulin is due to the same antibodies which have been demonstrated electrophoretically (13, 29). The finding that the binding of insulin could be demonstrated only in the sera of patients treated with insulin strengthens the conclusion of Berson, Yalow, Bauman, Rothschild, and Newerly (29) that the globulin responsible for this binding should be considered an antibody. It does not necessarily follow, however, that the affinity of insulin for the antibody is unchanged by the I^{131} label.

The lack of relationship between insulin binding and the insulin requirements of the patient is in apparent contradiction to the recent reports of Berson and Yalow (30, 33) and Burrows, Peters, and Lowell (34) that the sera of insulin resistant patients show much greater binding of insulin than do the sera of nonresistant patients. These authors found that the sera of insulin resistant patients bound 80 to 800 units of insulin per liter of serum, whereas nonresistant patients' sera rarely bound more than 10 units. Under the conditions of the present study (31), 100 per cent binding of the insulin would represent 66 units bound per liter of undiluted serum. Since the test was performed with a 1:25 dilution of serum, the insulin bound in undiluted serum would be higher than this because of the mass action effect. Under the conditions of the test, the serum of patients who had received insulin for more than five weeks bound from 2 to 56 per cent of the insulin, which represents 1 to 37 units of insulin bound per liter of serum. It is evident from this data that considerable binding of insulin may occur without marked insulin resistance. Unfortunately, no patient with an insulin requirement above 100 units per day was included in this study, since the data of Burrows and co-workers and Berson and associates suggest that unusually high binding capacities may contribute to insulin resistance. An alternative explanation of their data, given by Lazarow in the discussion following Reference 30, is that the high levels of antibody are the response to the large and repeated injections of insulin. Repeated determinations of insulin binding on the same patient through numerous changes in insulin requirement may help resolve this problem of which factor is cause and which is effect.

A partial explanation of the relative lack of effect of insulin antibody on the insulin requirements of the diabetic patient has been given by Yalow and Berson (35) in the inhibition of liver insulinase activity by serum containing insulin binding antibody. In contrast to the effect of antibody in accelerating antigen breakdown in some systems (36), the insulin antibody appears to retard antigen metabolism (29). Just as the

binding of insulin in a protamine precipitate does not increase total insulin requirement, the binding by antibody will not increase total insulin requirement if 1) the breakdown of the insulin is not accelerated, and 2) if either of the following obtains: *a*) the insulin-antibody bond is reversible, or *b*) the insulin-antibody complex is physiologically active. No information is available concerning the activity of the insulin-antibody complex, but evidence for the reversibility of the insulin-antibody bond has been given by Berson and Yalow (29) and by Skom and Talmage (31). Therefore, the two most apparent explanations for the relative lack of effect of antibodies on the insulin requirements of the patient are: 1) the failure of insulin antibodies to accelerate the metabolism of insulin, and 2) the reversibility of the insulin-antibody bond. As the free insulin is utilized by the cells and the free insulin concentration falls, the complex dissociates more rapidly than it forms, yielding more free insulin. As long as the binding of insulin is only temporary and does not lead to its accelerated metabolism, no increase in insulin requirements will result.

In contrast to the relative ineffectiveness of binding antibody in increasing the long term insulin requirement of the patient, binding antibody would be expected to increase the dose of insulin required for a given immediate effect. An example of this may be the increasing dosage of insulin required to induce shock in a schizophrenic patient receiving repeated injections. Berson and Yalow (30) suggest that the total effect of the insulin is not reduced by the presence of antibody in such cases but only delayed so that the hypoglycemia may be more prolonged.

SUMMARY

1. The insulin binding of the sera of 48 subjects has been determined.

2. Insulin binding was demonstrated in the sera of all 23 patients who had received insulin recently for more than one month.

3. Twelve control sera from patients who had never received insulin were all negative.

4. No consistent relationship could be demonstrated between the insulin binding and the insulin requirements of patients receiving insulin. The explanation of this relative lack of effect of insulin binding antibodies has been discussed.

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