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Studies on the Measurement of Insulin Antibodies in Controlled and Resistant Human Diabetic Patients by Polyacrylamide Gel Electrophoresis* (33980)

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During the past several years a number of methods have been developed for the assay of insulin and insulin antibody levels in biological materials (1–6). The nonspecific absorption of insulin to protein, and ^{131}I insulin isotope exchange between insulin and other proteins is a major drawback in many of the conventional immunological methods of assaying insulin and insulin antibody titers using ^{131}I insulin in biological materials.

To overcome this, we have now developed a new method by which the antibody or insulin levels in any given sample can be accurately and reproducibly determined without interferences as found in other techniques. For this we use the polyacrylamide analytical gel electrophoresis method of Ornstein (7) and Davis (8). The procedure is as follows: An insulin solution containing 800 mU/ml (80 mU of insulin-^{131}I, 720 mU of cold insulin) was prepared then diluted serially. To a series of tubes, each containing 0.3 ml of large-pore sample gel pH 6.6–6.8 and 1.5 mg of the unknown antiinsulin serum (AIS), 5–40 mU insulin contained in 0.05 ml of each dilution was then added. Glass columns $\frac{5}{16} \times 6$ in. containing 2.5 ml of polymerized 7% acrylamide gel (pH 8.6) and 0.2 ml of polymerized large-pore stacking gel were then prepared. A 0.2-ml sample of gel was then added to each column. The gels were electrophoreized for 1.5 hr using Tris–glycine buffer (pH 8.3) and 4 mA current/column. A standard curve was then drawn by using the serial dilutions and plotting cpm vs. mU. The exact amount of insulin added to each gel column was then calculated by subtracting the cpm of the remaining 0.1 ml from the cpm of starting 0.3 ml of gel and converting this to mU from the standard curve.

After electrophoresis, the gels were removed from the columns and cut in 0.5-mm slices. The sample gel, stacking gel, and 5 slices of 7% separating gel were then counted. The total cpm/gel were then converted to mU from the standard graph. A plot of mU taken vs. mU bound is then made. From this graph the potency of the AIS in mU/mg of serum may be calculated. With known potency, AIS, the procedure

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TABLE I. Effect of Increasing Concentration of Insulin and Time of Incubation on Insulin-Binding by GPAIS.

AIS (mg)	Insulin taken (mU)	Period of incubation	Insulin bound (mU)	Free insulin (mU)
1	5	30 min	4.5	0
1	10	30 min	9	0
1	15	30 min	12.1	1.6
1	20	30 min	12.7	4.7
1	40	30 min	13.6	21.6
1	50	1 hr	14	31
1	50	3 hr	14	29
1	50	6 hr	14	30

may be reversed and the amount of circulating free insulin in the serum may be calculated. This technique was very accurate in the μg range for assaying both AIS and insulin.

From the mobility of this insulin-binding protein on this 7% gel, we calculated its molecular weight to be around 150,000. This method is quantitative, and standard binding constants can be expressed graphically by varying either the amount of antibody taken or amount of subsaturating levels of insulin. The various parameters tested show (Table I) that incubation of insulin and antibody for 30 min at room temperature results in complete binding. Incubation up to 6 hr in cold does not alter the total amount of insulin bound. The distinct advantage of our method is that only the insulin bound to specific antibodies is measured and has no other interferences such as nonspecific binding, alcohol, and other absorbance induced dissociations. By using this polyacrylamide gel electrophoresis method we were able to detect insulin-binding antibodies in 15 out of

TABLE II. Insulin-Binding Antibodies in Diabetic Sera.

Patient	Clinical history		Antibody titer (mU of insulin bound/ml of serum)
	Blood sugar (mg/100 ml)	Clinical diagnosis and treatment	
R.D. 1	—	Insulin resistant	30.0
R.D. 2-M	—	Insulin resistant	28.0
N-1	—	Insulin resistant	10.5
T-1	—	Insulin resistant	5.0
U-1	—	Insulin resistant	4.8
No. 12	—	Insulin resistant	2.06
R.D. 0	—	Insulin resistant	1.7
No. 11	—	Insulin resistant	1.6
G-1	—	Insulin resistant	2.0
M-2	—	Insulin resistant	1.8
No. 16	176	Gout; uric acid 10 mg/100 ml	2.08
17	140	Gout; uric acid 9.9 mg/100 ml	0
4	500	36 U; ketoacidosis	3.03
13	190	70 UI + 10 U of reg.; juvenile onset	2.86
10	475	40 UI (pork)	2.08
15	170	DBI, 50 mg TD; and Diabinase, 500 mg	1.63
3	158	On Orinase	0
6	245	On Orinase, insulin for glucosuria	0
7	154	On Orinase	0
8	146	On Orinase	0
2	156	20 UI; diabetic	0
5	420	Juvenile onset; 35 U of cortisones	0
9	—	Insulinoma (islet cell tumor)	0
14	156	35 UI	0
Normal human serum (10 subjects)			0

16 resistant diabetic sera (Table II). As shown in Table II, the range of the antibody titer varied from 1.6–30.0 mU of insulin bound/ml of serum. Only one patient listed as resistant diabetic failed to show the presence of insulin antibodies. All these antibodies were in the same molecular weight range as found for the guinea pig antibody. No evidence of insulin antibodies were found in the sera of 3 controlled diabetics treated with Orinase as shown in Table II. One patient suffering from gout showed the presence of insulin antibodies while another did not (Table II). Sera from 10 normal persons failed to reveal the presence of any insulin antibodies. These results clearly demonstrate that there exists a close co-relationship between resistance to insulin therapy and the demonstrable presence of insulin antibodies. It is suggested that this new method could easily be utilized routinely for screening suspected diabetic patients for the detection of insulin antibody levels prior to choosing the type of preferred treatment.

Summary. A new method was developed by using polyacrylamide disc-gel electrophoresis for the assay of insulin and insulin antibodies in biological materials. By this method we have been able to detect insulin-binding antibodies in 15 out of 16 diabetic patients studied. The antibody titer ranged from 1.6 to 30.0 mU/ml of the serum. From the mobility of the insulin-binding protein (antibody) the molecular weight of this protein (both guinea pig and human and resistant diabetic) was around 150,000.

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