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SERUM INSULIN, INSULIN ANTIBODIES AND INSULIN REQUIREMENT IN THE FIRST PERIOD OF INSULIN TREATMENT IN NON-INSULIN RESISTANT DIABETICS*

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In the majority of patients insulin antibodies appear within weeks or months after the administration of insulin, and their presence interferes with the immunoassay of serum insulin. This difficulty can be circumvented in several ways: by ignoring the antibodies if their titer is low [12]; by separating free from bound insulin by alcohol [8] or on Sephadex columns [9] or by studying the patients in the first period of insulin treatment before the appearance of insulin antibodies [10, 11, 14]. The last approach seems at present to be the simplest and the best one. Under these conditions regular insulin radioimmunoassay does not differentiate between exogenous and endogenous insulin; separate endogenous insulin secretion can be measured as C-peptide immunoreactivity [4, 5].

Some cases of insulin resistance can be explained by the high titer and/or affinity of insulin antibodies. In the present work we tried to find out what influence, if any, the appearance of antibodies has on insulin requirement in non-resistant cases and what is the level of total immunoreactive insulin at the time of control of hyperglycemia before the appearance of antibodies.

MATERIAL AND METHODS

Twelve recently diagnosed diabetics aged 12 to 44 without any other known pathology were studied. None was obese. Six presented with severe ketonuria, the remaining 6 were not ketonuric but their fasting blood glucose level exceeded 250 mg/100 ml, OGTT showed almost no insulin reserve, and the trial with sulfonylureas and biguanides was unsuccessful.

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The patients were given mixed (predominantly bovine) NPH insulin in the morning. Blood assays were first performed 2-3 times a week, then (after 3 weeks) once a week and after 3 months, once a month. Blood glucose was determined both in the fasting state and post-prandially, the control of diabetes being accepted as good when these values did not exceed 160 mg/100 ml. Fasting blood specimens were assayed also for:

1) immunoreactive insulin. For patients on insulin the assay was performed against the bovine standard, though calibration curves for human and bovine insulin were virtually superimposable;

2) insulin binding by serum proteins: 0.1 ml of serum was mixed with 0.1 ml of ^{125}I-insulin (25 μU) and incubated for 24 hrs at 4 °C. Free and antibody-bound insulin were separated on Sephadex G-100 columns at pH 7.4. Preliminary experiments showed that labelled insulin (Amersham, England) after fractionation on Sephadex showed 4-6% of radioactivity present in the high molecular fraction. The same was true when labelled insulin was incubated with non-immune serum. When insulin antibodies appeared, label binding by the protein fraction increased to at least 20-30%;

3) total insulin was assayed after dissociation of the insulin-antibody complexes on Sephadex G-100 columns at pH 3.0 as previously described [9];

4) total insulin binding capacity was determined according to BERSON and YALOW [2] but instead of whole serum we used the bound insulin free protein fraction.

Sudden increase of immunoreactive insulin to levels outside the standard curve (above 200 μU/ml), appearance of insulin binding by serum proteins and the total insulin level reaching hundreds and even thousands of μU/ml were considered as indicative of the presence of insulin antibodies. All the parameters showed simultaneous changes.

RESULTS

1) Immunoreactive insulin - In 4 out of 12 patients control was achieved after the appearance of insulin antibodies. The results in the remaining 8 patients (fig. 1) showed virtually no change at the time when blood glucose in the fasting state dropped to almost normal level.

2) Insulin antibodies (fig. 2) - Out of 12 patients, in 4 no antibodies were found after 200 days (and in 2 even after 500 days) of insulin treatment. In others, the antibodies appeared after 10-87 days. It is remarkable that in 2 patients they appeared already after 10 and 12 days of insulin treatment. In all these patients with antibodies the maximum insulin binding capacity very quickly (within 1-2 weeks after the first appearance of antibodies) reached the level above which it did not increase further.

3) Insulin antibodies and insulin requirement - As can be seen from fig. 2, out of 8 cases with insulin antibodies, in 6 there were virtually no changes in insulin requirement (neither increase nor decrease by more than 8 U/*die*), in 2 patients insulin requirement decreased by 20 and 24 U/*die* respectively. No relation between insulin binding capacity and insulin dose was seen. The total insulin was 200-3,200 μU/ml (see tab. 1) which constituted only 3 to 36% of the maximum insulin binding capacity (4-12 U/l).

DISCUSSION AND CONCLUSIONS

There is reason to believe that immunoreactive insulin determined in the serum of patients 24 hrs after the last injection of insulin was in fact their endogenous insulin, since elevation of serum insulin after the administration

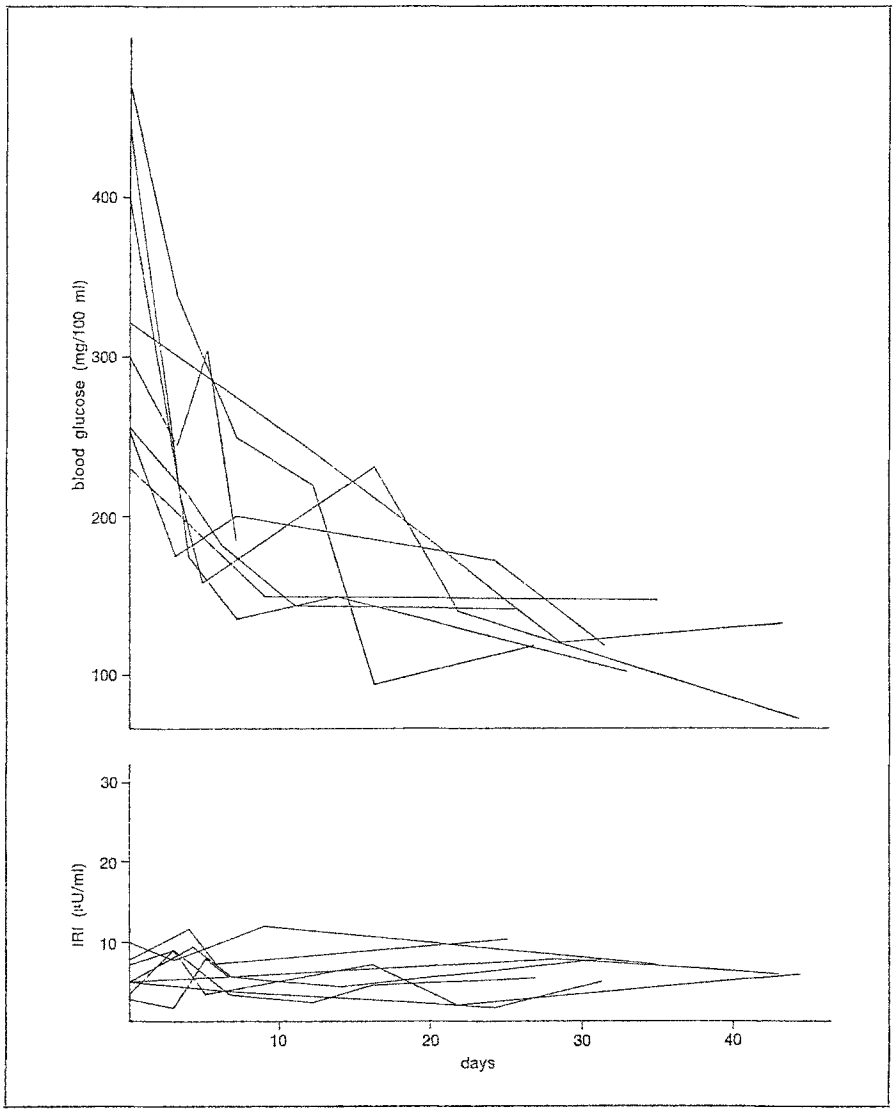

Fig. 1 - Serum immunoreactive insulin (IRI) and blood glucose in the fasting state in the first period of treatment by mixed beef-pork NPH insulin before the appearance of insulin antibodies.

of NPH insulin lasts much less [7], and because the determination of C-peptide immunoreactivity proved the preservation of residual insulin secretion in the first period of insulin treatment [4, 5]. The seemingly paradoxical lack of change of immunoreactive insulin at the time of the rather sharp drop in hyperglycemia can be explained in the following way. In the uncontrolled diabetic, derangements in carbohydrate metabolism during the day are of such a magnitude that the residual insulin secretion is able to bring the

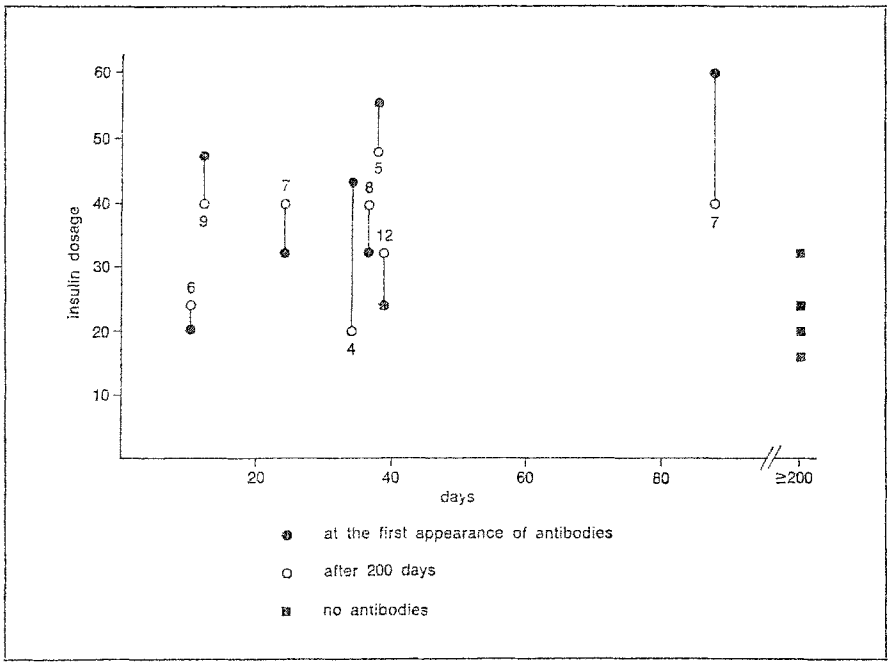

Fig. 2 - Time of first appearance of insulin antibodies and insulin dosage at the time of their appearance or after 200 days of treatment by commercial NPH insulin. Numbers indicate in each case the maximum insulin binding capacity in U/l after 200 days of insulin treatment.

case no.	insulin dosage (U/*die*)	maximum insulin binding capacity (U/l)	total serum insulin (μU/ml)	total serum insulin as % of maximum binding capacity	calculated daily insulin wastage (μU/ml)
1	20	4	700	18	1.2
2	24	6	1,700	28	3.0
3	32	12	2,200	18	3.8
4	40	7	700	10	1.2
5	40	7	200	3	0.35
6	40	8	2,400	30	4.2
7	40	9	3,200	36	5.6
8	48	5	1,050	20	1.8

Table 1 - Insulin requirement, maximum insulin binding capacity and total insulin in serum after 200 days of insulin treatment (all patients except case 1 were ketotic prior to insulin treatment).

hyperglycemia down to some extent during the night but not to the normal level. In patients well controlled by exogenous insulin the latter prevented hyperglycemia during the day, and even insufficient endogenous insulin secretion was enough to prevent increase of blood glucose at night. This suggestion can explain the lack of a reciprocal relationship between immunoreactive insulin and blood glucose in insulin-treated patients [11, 14].

The difference in the time of appearance of antibodies was remarkable — from 10 days to more than 500 days, irrespective of insulin dose. Therefore, it is not correct to presume that every patient treated with insulin for less than 2 weeks has no antibodies [11] or that everyone treated for several months does have them. Every patient requires individual antibody determination, and insulin dosage and its changes provide no clues as to the presence or absence of antibodies. In this our data correspond to those of SCHLICHTKRULL [15] who found that after one year of treatment by conventional insulins some of the patients had no insulin antibodies.

The estimation of insulin wastage caused by the destruction of the insulin-antibody complexes is based on the assumptions that antibody distribution space is about 7 l and that plasma clearance of antigen-antibody complexes is about 25% per day. BERSON and YALOW [3] calculated that in most cases insulin wastage did not exceed 10 to 15 U/*die*. Our data are still more conservative, since in our cases only 3 to 36% (mean 20%) of the maximum insulin binding capacity was occupied by insulin, and the daily insulin wastage was negligible (0.35 to 5.6 U/*die*). In other words, in insulin-sensitive cases insulin antibodies exert very negligible influence on insulin requirement. This finding is in agreement with the observations according to which switching of patients from commercial preparations to monocomponent insulin resulted in substantial decrease in antibodies, but decrease in insulin requirement was neither very significant nor constant [1, 6].

Insulin antibodies play undoubtedly an important clinical role prolonging the action of injected insulin [13], but this effect can be even beneficial and may lead to a decrease in insulin requirement, which might have happened in two of our patients whose insulin requirement substantially decreased after the appearance of insulin antibodies.

SUMMARY

Twelve insulin-sensitive diabetics were studied for 200 days after the initiation of mixed beef-pork NPH insulin. Normalization of the fasting blood glucose was not accompanied by any elevation in the pre-treatment fasting immunoreactive insulin level. Insulin antibodies appeared in 2 patients on the second week of insulin treatment, in 6 others within 87 days. In 4 patients no antibodies were found 200 days after the start of insulin. The appearance of antibodies was accompanied in two patients by a decrease in insulin requirement, in others there was no change. When antibodies were present, the total maximum insulin binding capacity was 4 to 12 U/l, but the total insulin constituted only 3 to 36% of the binding capacity. Insulin wastage caused by the destruction of the immune complexes was calculated to be 0.35 to 5.6 U/*die* only, and this explains the negligible effect of insulin antibodies on insulin requirement in non-resistant patients.

REFERENCES

1) ANDREANI D., IAVICOLI M., TAMBURRANO G., MENZINGER G., MALTARELLO C.: Comparative Trials with Monocomponent (MC) and Monospecies (MS) Pork Insulins in the Treatment of Diabetes Mellitus. Influence on Antibody Levels, on Insulin Requirement and on Some Complications - Hormone metab. Res. *6*, 447, 1974.

2) BERSON S. A., YALOW R. S.: Quantitative Aspects of the Reaction between Insulin and Insulin-Binding Antibody - J. clin. Invest. *38*, 1996, 1959.
3) BERSON S. A., YALOW R. S.: Insulin 'Antagonists' and Insulin Resistance - In: ELLENBERG M., RIFKIN H. (Eds): Diabetes Mellitus: Theory and Practice. McGraw-Hill Book Co., New York, 1970; p. 388.
4) BLOCK M. B., MAKO M. E., STEINER D. F., RUBENSTEIN A. H.: Circulating C-Peptide Immunoreactivity. Studies in Normals and Diabetic Patients - Diabetes *21*, 1013, 1972.
5) BLOCK M. B., ROSENFIELD R. L., MAKO M. E., STEINER D. F., RUBENSTEIN A. H.: Sequential Changes in Beta-Cell Function in Insulin-Treated Diabetic Patients Assessed by C-Peptide Immunoreactivity - New Engl. J. Med. *288*, 1144, 1973.
6) CZYŻYK A., ŁAWECKI J., ROGALA H., MIEDZIŃSKA E., POPIK-HANKIEWICZ A.: Serum Levels of Insulin-Binding Antibodies in Diabetic Patients Treated with Monocomponent Insulin - Diabetologia *10*, 233, 1974.
7) GINSBERG S., BLOCK M. B., MAKO M. E., RUBENSTEIN A. H.: Serum Insulin Levels Following Administration of Exogenous Insulin - J. clin. Endocr. *36*, 1175, 1973.
8) HEDING L. G.: Determination of Free and Antibody-Bound Insulin in Insulin-Treated Diabetic Patients - Hormone metab. Res. *1*, 145, 1969.
9) LEV-RAN A., RATT L.: Insulin Binding and Free Insulin in Serum of Insulin-Treated Diabetics - Hormone metab. Res. *5*, 67, 1973.
10) METZ R., BOWERS M.: Serum Insulin in Insulin-Treated Diabetics - Diabetes *20*, 368, 1971.
11) METZ R., BOWERS M.: Plasma Insulin, Glucose and Free Fatty Acids Relationships in Insulin-Treated Diabetics - Diabetes *21*, 325, 1972.
12) MOLNAR G. D., TAYLOR W. F., LANGWORTHY A. L.: Plasma Immunoreactive Insulin Patterns in Insulin-Treated Diabetics - Mayo Clin. Proc. *47*, 709, 1972.
13) PALUMBO P. J., TAYLOR W. F., MOLNAR G. D., TAUXE W. N.: Disappearance of Bovine Insulin from Plasma in Diabetic and Normal Subjects - Metabolism *21*, 787, 1972.
14) QUICKEL K. E., FELDMAN J. M.: Serum Insulin Levels in Patients on Insulin - Lancet *2*, 212, 1971.
15) SCHLICHTKRULL J.: Monocomponent Insulin and its Clinical Implications - In: BASTENIE P. A., GEPTS W. (Eds): Immunity and Autoimmunity in Diabetes Mellitus. Excerpta Medica, Amsterdam/American Elsevier Publ. Co., Inc., New York, 1973; p. 29.
16) WALDHÄUSL W.: Insulinbindende Antikörper und Insulinresistenz - Wien. klin. Wschr. *85*, 833, 1973.

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