The Insulin Content of Blood Plasma

J. Bornstein, M.D.

ST. LOUIS, MISSOURI

Gellhorn and Feldmann[1] in 1941, using adrenomedullated hypophysectomized rats, found that the plasma of fed dogs caused a profounder degree of hypoglycemic than that of fasted dogs, the apparent insulin concentration being approximately doubled. Although Gellhorn and Feldman's estimates of insulin concentration are in the same range as we have found, the technic is liable to the criticism that as the test animals' beta cells were not destroyed, the rats' own insulin may have interfered with the assay.

In 1947 Anderson, Lindner, and Sutton[2] published the details of a technique using ADH (adrenomedullated, alloxan diabetic, hypophysectomized) rats. Using these preparations Anderson was able to demonstrate that a perfused pancreas secreted insulin in response to a rise in the glucose concentration of the perfusate, and that the addition of growth hormone interfered in some way with the assayable insulin. An action of growth hormone in the pancreas has also been demonstrated by Bornstein, Reid and Young.[3] The sensitivity of this technic was approximately 1/8000 units of insulin or 0.125 milliunit. In 1948, one of us (J. B.) repeated the work of Anderson with a view to attempting to assay insulin in human plasma. A similar degree of sensitivity was obtained and it was found that if glucose were administered to a dog, blood obtained from the pancreatic vein had an increased insulin concentration. When work commenced with human plasma, we found that the administration of 0.5 to 1.0 ml. plasma was required to produce a hypoglycemic response. The administration of this volume of plasma intravenously to nembutalized ADH rats frequently caused death due to acute cardiorespiratory failure. Accordingly we re-examined the technic with a view to carrying out the assay over a longer period of time and giving the substances under test subcutaneously. It was found by varying the environmental conditions that it was possible to induce a state where the blood glucose concentration varied very little over a period of one hour, and accordingly an assay was constructed using this period of time. The range of sensitivity was from 1/20,000 to 1/2,000 of a unit of insulin (0.05 to 0.5 milliunit).

Here I should like to point out that in my opinion the only reason for the apparent increase in sensitivity is that the assay is carried out on a relatively stable base, whereas Dr. Anderson's assay is obtained on a rising base line. The curves obtained with both technics are very similar. The real advantage of this modification is that larger amounts of plasma can be administered and anesthesia and surgery during the assay are avoided.

From the National Health and Medical Research Council of Australia Biochemistry Dept., University of Melbourne.

Presented at the meeting of the American Diabetes Association, Chicago, June 8, 1952.

Address communications to Dr. Bornstein, Washington University, Department of Biological Chemistry, Euclid Avenue and Kingshighway, St. Louis 10, Missouri.

This technic was then applied to the study of plasma insulin concentration in normal man under the conditions of a glucose tolerance test. It was found that the detectable plasma insulin increased with the rise in blood sugar, reaching a peak when the glucose had been restored to normal and thereafter declining. The details of controls required for such an assay have been published elsewhere.[4] The technic was next applied to the study of diabetic patients. As it had previously been found that in the normal individual, insulin was at an apparent peak two hours after glucose, this period was arbitrarily chosen for the studies in cases of diabetes.

Altogether a control group of 14 normal persons of all ages and 28 "new" cases of diabetes were studied. It was found that the concentration of available plasma insulin of the normal controls varied from 0.24 to 0.40 milliunit per ml. with a mean of 0.34 mu/ml. whereas the cases of diabetes fell into two groups, one group of 15 cases in which no insulin could be detected showed considerable loss of weight and some degree of ketosis as principal clinical characteristics. The other group of 13 cases, in which a range of 0.10 to 0.32 milliunit per ml. was found (with a mean of 0.23 milliunit per ml.), was characterized by the presence of obesity, lack of ketosis and symptoms of various complications of diabetes mellitus rather than those of the syndrome itself. It is interesting to note that the group in which no insulin could be detected included patients of all ages from 5 to 64 which with a mean age of 36, the patients in the second group being preponderantly female, largely over middle age, (an average age of 49.) This leads us to believe that the mild form of diabetes is largely confined to women in the later years of life. These older obese patients in nearly all cases could be stabilized without administration of insulin.

A number of other cases have been studied. Four of the patients showing no insulin in their plasma were studied after they had been stabilized on diet and insulin. The blood was taken at a time when the blood sugar was found to be within the normal range. The plasma insulin under these conditions was found to be about 0.25 milliunit per ml.

Tests were made in two cases showing marked insulin resistance. It was necessary to give over 2000 units per day to depress the blood sugar significantly. In each case blood was taken 1 hour after the injection of 300 units of insulin. No insulin could be detected.

One case of hyperinsulinism with islet carcinoma was studied but an attempt to estimate the insulin concentration in the blood was unsuccessful, as 0.5 ml. of the plasma produced rapidly fatal hypoglycemia in the test animals. In three cases of spontaneous hypoglycemia after gastrectomy, the patients had as one of the major characteristics, normal sensitivity to insulin but a failure of the blood sugar to recover in a normal time, when an insulin tolerance test was performed. Here, glucose was administered and blood samples were taken at 1 and 2 hours; the hypoglycemic attack usually developed at these times. The peak range of insulin concentration was from 0.64 to 0.86 milliunit per ml.

At this stage it must be pointed out that such an assay does not absolutely measure the amount of insulin present but merely detects the excess of insulin over its antagonists absorbed in unit time. Accordingly the finding of a zero value for insulin does not necessarily mean that the patient is failing to secrete the hormone but may merely mean that inhibitors are present in sufficient excess in the plasma to mask completely any insulin effect.

Evidence for these views came to light when we found that following an injection of the plasma of some diabetic patients with no apparent insulin in the plasma there was a marked decrease in the insulin sensitivity of the ADHA (alloxan-diabetic, hypohysectomized adrenalectomized) rats used in the assays. Further evidence demonstrating the presence of a circulating inhibitor in plasma has recently been obtained by Bornstein and Park in the Department of Biological Chemistry, Washington University, St. Louis. Using a specially modified technic of handling the rat diaphragm it has been found that in the presence of the serum of the diabetic rat, the rate of utilization of glucose is depressed as compared with the serum from the fasting normal rat and that this inhibitory action of the serum is abolished by either hypophysectomy or adrenalectomy of the donor animal. Replacement therapy with growth hormone alone or cortisone alone fails to restore the inhibition but the administration of both restores the inhibition; in all cases the inhibition, when present, is reversible by insulin provided a sufficient concentration of insulin is used. It is of interest that the inhibiting substance once present is extremely persistent. In groups of diabetic hypophysectomized rats injected with growth hormone and cortisone the inhibitor was fully present seven days after the last injection of the hormones. This finding we feel is analogous to the finding in the ADHA rats in which the inhibition persisted for varying periods after the injection of plasma but could always be abolished by increasing the dose of insulin. The nature and site of action of the inhibitory substance or substances is at present unknown.

It is hoped that the recent work of Rabin and his coworkers in apparently separating the diabetogenic principle from the growth principle in pituitary extracts may yield information of value in this respect.

SUMMARY

In conclusion I should like to state that in my opinion the syndrome of diabetes mellitus is due to an imbalance between insulin and the pituitary-adrenal axis and thus may be caused by either failure to secrete insulin or the hyperfunction of the pituitary adrenal axis, although at present other factors cannot be eliminated.

REFERENCES

[1] Gellhorn, E.; Feldman, J.; and Allen, A.: Assay of insulin on hypophysectomised, adrenomedullated rats. Endocrinology *29*:137, 1941.

[2] Anderson, Evelyn; Lindner, Erna; and Sutton, Virginia: A sensitive method for the assay of insulin in blood. Am. J. of Physiol. *149*:350, 1947.

[3] Bornstein, J.; Reid, E. and Young, F. G. The hyperglyties of insulin using alloxan diabetic, hypophysectomised adrenalectomised rats. Australian J. Exper. Biol. & M. Sc. *28*:87-91, 1950.

[4] Bornstein, J.: A technique for the assay of small quanticemic action of blood from animals treated with growth hormone. Nature *168*:903-907, 1951.

[5] Bornstein, J., and Lawrence, R. D., Plasma insulin in human diabetes mellitus. Brit. M. J. *II*:1541-1548, 1951.

[6] Bornstein, J., and Trewhella, P., An anti-insulin factor in the plasma of diabetic patients. M. J. Australia. p. 119-128, January, 1951.

DISCUSSION

DR. FRANCIS D. W. LUKENS (*Philadelphia, Pa.*): There has been a great deal of interest in Dr. Bornstein's work, and it is a privilege to have him here and to learn of this at first hand. One cannot discuss all the technical details of these very difficult methods, but I might say that over the years, and with the stimulation that Dr. Best has given to many of us, a great many people have been trying to measure insulin in blood, and these efforts are constantly improving. This is at least a very important step forward, regardless of how much it may be refined in the future.

Whenever a new method is developed and whenever new facts are observed, our old ideas must be adjusted. Here is one thought that Dr. Bornstein's report gave me. Dr. Wrenshall has reported to this society on the fairly consistent low insulin content of the pancreas of young diabetics; supposedly it is a form of insulin-deficient diabetes. Drs. Falta, Himsworth, Lawrence and others have commented on the insulin sensitivity of young diabetics and have thought this might mean an insulin-deficient diabetes. If I understood him, Dr. Bornstein said that what may be called "zero insulin diabetics" (I must paraphrase him hastily) fall into all age groups. Will this force us to realign some of our present understanding? In any case Dr. Bornstein's experiments will stimulate our thinking considerably.

DR. J. BORNSTEIN, (*Closing*): I do not consider that Dr. Lukens need be quite so concerned about this apparent discrepancy, because in our study, we have unfortunately been able to study only three patients under the age of twenty. In these cases no insulin could be detected, and the plasma did not diminish the subsequent insulin sensitivity of the animals.

The other cases, in which a zero value for insulin was obtained, are only regarded as an apparent lack of insulin, as in all cases the insulin sensitivity of the test animals was diminished after the injection of the test plasma, and so I would interpret these findings as indicating that probably a large excess of inhibitory substance was probably present, and that this masks any insulin effect, although insulin may be present in such a sample of plasma.