Brief Notes and Comments

Hemoglobin A_{Ic} as an Indicator of the Degree of Glucose Intolerance in Diabetes

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SUMMARY

Hemoglobin A_{Ic} concentration, fasting blood sugar, response to an oral glucose tolerance test, and skeletal muscle capillary basement membrane thickness were measured in diabetic patients. Hemoglobin A_{Ic} concentration correlates with both response to a glucose tolerance test ($r = 0.82$, $p < 0.001$) and fasting blood sugar ($r = 0.62$, $p < 0.001$). The correlation of hemoglobin A_{Ic} concentration with glucose tolerance is independent of fasting blood sugar concentration (partial $r = 0.61$, $p < 0.005$), whereas that of hemoglobin AIc with fasting blood sugar probably reflects the relationship between fasting blood sugar levels and glucose tolerance (partial $r = 0.22$, $p > 0.05$). Hemoglobin A_{Ic} levels do not correlate with basement membrane thickness ($r = 0.15$, $p > 0.05$). DIABETES *25*:230-32, March, 1976.

Although the abnormalities of glucose metabolism are well defined in diabetes mellitus, it has been difficult to correlate these abnormalities with the major secondary sequelae of the disease, including micro- and macroangiopathy. We have recently initiated studies of the glycohemoglobin, hemoglobin A_{Ic}, in hopes of gaining insight into the pathologic sequelae of diabetes mellitus.

Hemoglobin A_{Ic} (Hb A_{Ic}), which comprises about 3-6 per cent of normal human hemoglobin, is elevated to 6-12 per cent in diabetes.[1] Hemoglobin A_{Ic} differs from hemoglobin A in that an unknown carbohydrate is attached to the amino-terminal valine of the β-chain.[2] Studies in the diabetic mouse[3,4] have shown a similarly elevated hemoglobin that is made as a postsynthetic modification of hemoglobin A at a constant rate throughout the life of the red cell. The rate of synthesis of hemoglobin A_{Ic} appears to be dependent on the presence of an unknown plasma factor present in elevated amounts in mice with either genetic or chemically induced diabetes after they become hyperglycemic. Hemoglobin A_{Ic} production is an example of the modification of a naturally occurring protein by the addition of a carbohydrate moiety that results in altered characteristics—e.g., electrophoretic mobility[5] and oxygen affinity.[6] Similar glycosylation reactions might explain many of the secondary abnormalities in diabetes, including white cell dysfunction,[7] platelet dysfunction,[8] and basement membrane thickening,[9] as well as abnormal structure or function of other specific proteins—for example, lens[10] or peripheral nerves.[11]

In an attempt to correlate hemoglobin A_{Ic} with the microangiopathy of diabetes, hemoglobin A_{Ic} was measured in normal subjects and in diabetics, with and without basement-membrane thickening. Glucose tolerance tests and measurements of skeletal muscle capillary basement-membrane thickness, in addition to fasting blood-sugar determinations, were performed in subgroups of those subjects.

MATERIALS AND METHODS

A total of 38 diabetic patients, ranging in age from 16 to 82 years, were studied. Seventeen were maintained on insulin therapy, 12 on oral hypoglycemic agents, and nine on diet alone. Thirteen patients were females; 25 were males.

Muscle capillary basement-membrane thickness was determined in 21 of these patients as described previously.[12] Hemoglobin A_{Ic} was isolated in dupli-

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cate by the column chromatographic method of Trivelli et al.[1] and quantified by the method of Drabkin.[13]

Fasting blood sugars were measured on at least two occasions in 33 patients. Oral glucose tolerance tests were performed by giving a 75-gm. load of glucose after an overnight fast. Glucose concentrations were determined by an AutoAnalyzer. Ten nondiabetic controls, as judged by normal responses to the oral glucose load,[14] were also studied. The response to the glucose challenge was determined by measuring the area under the curve by planimetry of a three-hour glucose tolerance test. These studies were approved by the Clinical Review Boards of the appropriate institutions.

RESULTS

Glucose tolerance tests were performed on 22 diabetic patients. In this group, hemoglobin A_{Ic} levels varied from 4.49 per cent to 11.20 per cent. The peak glucose response to the glucose tolerance test ranged from 184 mg./dl. to 880 mg./dl. The correlation coefficient between these two variables is highly significant ($r = 0.82$, $p < 0.001$) (figure 1). The results are unaltered by utilizing the areas under the glucose tolerance curves ($r = 0.76$, $p < 0.001$) instead of the peak values. A significant but less impressive correlation was also found between fasting blood sugar and hemoglobin A_{Ic} levels in the 33 diabetics studied ($r = 0.62$, $p < 0.001$) (figure 2). Because fasting blood sugar concentrations and response to a glucose tolerance test are interdependent variables that correlate well with each other ($r = 0.77$, $p < 0.001$), their respective correlations with hemoglobin A_{Ic} concentration might simply reflect their interdependence. Partial correlation coefficients,[15] which take into account the interdependence of response to a glucose tolerance test and fasting blood sugar, revealed that hemoglobin A_{Ic} concentration and response to a glucose tolerance test are indeed correlated (partial $r = 0.61$, $p < 0.005$); whereas the correlation between fasting blood sugar and hemoglobin A_{Ic} concentrations loses its significance (partial $r = 0.22$, $p > 0.05$). The ten nondiabetic controls had hemoglobin A_{Ic} concentrations ranging from 3.97 per cent to 5.04 per cent, and peak values of 73 mg./dl. to 216 mg./dl. during the oral glucose tolerance test. There was no correlation between hemoglobin A_{Ic} and glucose tolerance in this population ($r = -0.43$, $p > 0.05$).

Muscle capillary basement-membrane thickness varied from 612 ± 141 to 2,975 ± 830 A in the 21 diabetics studied. The hemoglobin A_{Ic} concentrations of these same patients ranged from 4.49 per cent to 10.84 per cent. The correlation coefficient obtained

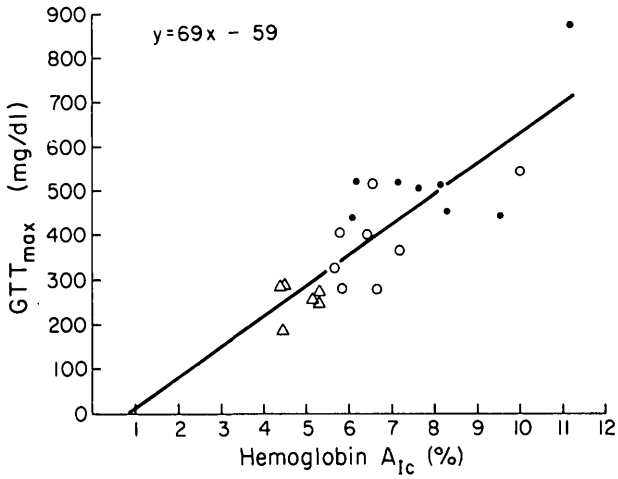

FIG. 1. Correlation between hemoglobin A_{Ic} concentration and maximal response to an oral glucose tolerance test in 22 diabetics (● patients treated with insulin, o patients treated with oral hypoglycemic agents, Δ patients treated with diet alone).

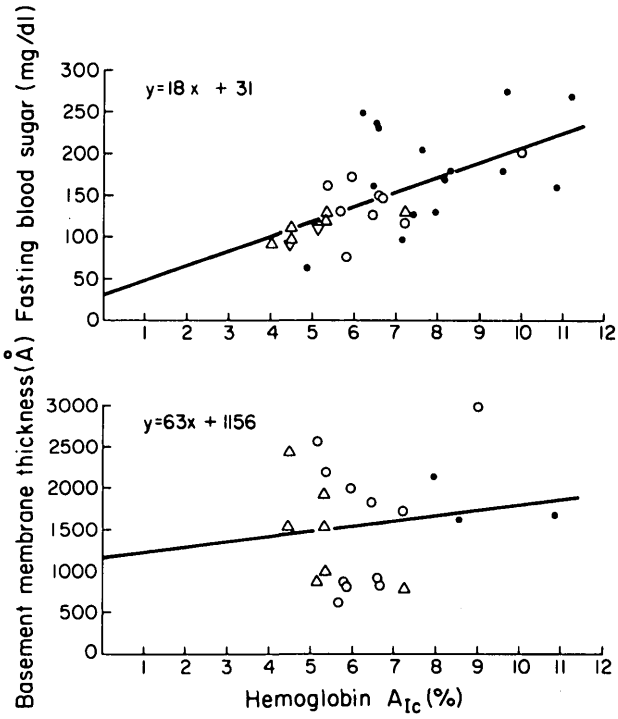

FIG. 2. Correlations between hemoglobin A_{Ic} levels and both fasting blood sugar (top) and skeletal muscle capillary basement membrane thickness (bottom) in diabetic patients (● patients treated with insulin, o patients treated with oral hypoglycemic agents, Δ patients treated with diet alone).

between these two parameters was not statistically significant ($r = 0.15$, $p > 0.05$) (figure 2).

DISCUSSION

Although a previous study[1] failed to demonstrate a correlation between the degree of elevation of "fast" hemoglobins (which include Hb A_{Ic}) and clinical parameters of diabetes, we report a highly significant correlation ($r = 0.82$, $p < 0.001$) between Hb A_{Ic} concentration and response to an oral glucose tolerance test in diabetic patients. This correlation is independent of fasting blood sugar levels, as determined by the partial correlation coefficient (partial $r = 0.61$, $p < 0.005$). Although a significant correlation was found between hemoglobin A_{Ic} and fasting blood sugar concentrations ($r = 0.62$, $p < 0.001$), this correlation probably reflects the interdependence of fasting blood sugar and glucose tolerance, since the partial correlation coefficient, which takes this interdependence into account, revealed no correlation between fasting blood sugar and Hb A_{Ic} concentrations (partial $r = 0.22$, $p > 0.05$). Hemoglobin A_{Ic} levels probably reflect, better than fasting blood sugar concentrations or glucose tolerance test results, the mean daily blood sugar concentration and the degree of carbohydrate imbalance, and may provide a better index of control of the diabetic patient without resorting to a glucose loading procedure.

The lack of correlation of hemoglobin A_{Ic} levels with muscle capillary basement-membrane thickness is disappointing, since the thickening of basement membrane is believed to be intimately related to the microangiopathic sequelae of diabetes. This lack of correlation may be due either to the fact that basement-membrane thickening is probably the result of kinetic and chemical factors, some of which are unrelated to events involved in Hb A_{Ic} synthesis, or to the fact that the hemoglobin A_{Ic} levels measured reflect the degree of control over only the previous few months, whereas several years are required for the development of detectable basement-membrane thickening.

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REFERENCES

[1]Trivelli, L.A., Ranney, H.M., and Lai, H.-T.: Hemoglobin components in patients with diabetes mellitus. N. Engl. J. Med. *284*:353-57, 1971.

[2]Bookchin, R.M., and Gallop, P.M.: Structure of hemoglobin A_{Ic}: Nature of the N-terminal β-chain blocking group. Biochem. Biophys. Res. Commun. *32*:86-93, 1968.

[3]Koenig, R.J., and Cerami, A.: Synthesis of hemoglobin A_{Ic} in normal and diabetic mice: A potential model of basement membrane thickening. Proc. Natl. Acad. Sci. U.S.A. *72*:3687-91, 1975.

[4]Koenig, R.J., Araujo, D.C., and Cerami, A.: Increased hemoglobin A_{Ic} in diabetic mice. Diabetes *25*:1-5, 1976.

[5]Rahbar, S., Blumenfeld, O., and Ranney, H.M.: Studies of an unusual hemoglobin in patients with diabetes mellitus. Biochem. Biophys. Res. Commun. *36*:838-43, 1969.

[6]Bunn, H.F., and Briehl, R.W.: The interaction of 2,3-diphosphoglycerate with various human hemoglobins. J. Clin. Invest. *49*:1088-95, 1970.

[7]Perillie, P.E., Nolan, J.P., and Finch, S.C.: Studies of the resistance to infection in diabetes mellitus. Local exudative cellular response. J. Lab. Clin. Med. *59*:1008-15, 1962.

[8]Bensoussan, D., Levy-Toledano, S., Passa, P., Caen, J., and Canivet, J.: Platelet hyperaggregation and increased plasma level of Von Willebrand factor in diabetics with retinopathy. Diabetologia *11*:307-12, 1975.

[9]Beisswenger, P.J., and Spiro, R.G.: Studies on the human glomerular basement membrane. Composition, nature of the carbohydrate units and chemical changes in diabetes mellitus. Diabetes *22*:180-93, 1973.

[10]Bradley, R.F., and Ramos, E.: The eyes and diabetes. *In* Joslin's Diabetes Mellitus. Marble, A., White, P., Bradley, R.F., and Krall, L.P., editors. Philadelphia, Lea & Febiger, 1971, pp. 478-525.

[11]Fagerberg, S.-E.: Diabetic neuropathy. Acta Med. Scand. Suppl. *345*, 1959.

[12]Kilo, C., Vogler, N., and Williamson, J.R.: Muscle capillary basement membrane changes related to aging and to diabetes mellitus. Diabetes *21*:881-905, 1972.

[13]Drabkin, D.L.: The standardization of hemoglobin measurement. Am. J. Med. Sci. *217*:710-11, 1949.

[14]Steinke, J., and Thorn, G.W.: Diabetes mellitus. *In* Harrison's Principles of Internal Medicine. Wintrobe, M.M., Thorn, G.W., Adams, R.D., Braunwald, E., Isselbacher, K.J., and Petersdorf, R.G., Eds. New York, McGraw-Hill, 1974, pp. 538-39.

[15]Hays, W.B.: Statistics. New York, Holt, Rinehart and Winston, 1963, pp. 574-76.