Progress Report

TITLE: High Performance Liquid Chromatography of Hemoglobin A_{1C} and Other Glycosylated Hemoglobins on a Weak Cation-Exchange (1-85-0014)

Investigator: Ching-Nan Ou, Ph.D.
Department of Pathology
Baylor College of Medicine and
Texas Children's Hospital

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A liquid chromatographic method using a poly (aspartic acid) cation exchange column is developed for the quantitation of hemoglobin AIC and for the analysis of human hemoglobins. The gradient elution programs use the following mobile phrases: A:40 mM Bis-Tris, 4 mM KCN (pH 6.5); B:40 mM Bis-Tris, 4 mMKCN, 0.2 M NaCl (pH 6.8). With the flow rate of 1 ml/min, the column is equilibrated with mobile phase consists of 10% B. Elution of hemoglobins is accomplished by increasing the percent of B buffer to 56% and 100% at 16 and 18 min, respectively. The percent of B buffer is then maintained at 100% for 5 min and decreased to 10% within 2 min. After re-equilibration with 10% B buffer for 5 min the next sample is then injected. Comparison of the method with ion exchange column chromatography (Isolab) yields the following results: y (HPLC) = 0.96 x (Isolab method) + 0.16, r = 0.949, n = 89 and $S_{y \cdot x}$ = 0.87. The day-to-day precision of the assay is less than 3%. The commonly encountered abnormal hemoglobins such as S.C.D.E.O.G.SG. can be easily identified without further confirmation by other methodology.

The high resolution of the system and simplicity of the method plus the automation make the procedure useful for the accurate measurement of hemoglobin A_{1C} and screening of common hemoglobin disorders.

The interference of hemoglobin F with AIC is still under investigation. The investigation supported by this grant has resulted in an abstract presented at the 37th National Meeting of American Association for Clinical Chemistry, at Atlanta, Georgia, July 1985 and published in Clinical Chemistry 31, 945 (1985).

The mean percent difference in G-albumin measured after glucose removal was + 29.1%. The change in G-albumin levels could not be explained by glucose levels as evidenced by a r < 0.4 found on analysis of the data sets. Therefore, our studies suggest that glucose should, unless shown otherwise, be removed before affinity column separation. Also, G-IgG may prove to be a better indicator of diabetic plasma protein glycation than albumin due an apparent greater sensitivity to glycation.

220 CLINICAL AND ANALYTICAL STUDIES OF GLYCATED HEMOGLOBIN AND GLYCATED ALBUMIN ASSAY BY AFFINITY CHROMATOGRAPHIC METHODS Pennell Painter, Jonn Evans, William Law, John Eaddy, June Cope, and Jane Smith (Univ. of Tennessee Memorial Hospital, Knoxville, TN 37920). (Spon.: Burton Goodge)

We report some of the results from our 18 months experience with the Pierce Chemical Co. Glyco-Gel™ (1.0 mL) and Isolab Inc. GlycAffin™ (0.5 mL) affinity chromatography methods for measurement of glycated proteins. All analyses were carried out at 27°C using vendor protocols. The albumin concentration from column collections was measured immunologically on the Beckman Immunochemistry System (ICS™) and by the vendor's bromcresol green (BCG) dye binding method. Glycated hemoglobin (G-Hb%) and albumin (G-Alb%) levels measured on 100 patient samples following separation on the two columns showed r's > 0.990.

Plasma pool precision studies	1.0ML Pierce N=20				0.5ML Isolab N=20			
	Within-run		Between-day		Within-run		Between-day	
	Mean	%CV	Mean	%CV	Mean	%CV	Mean	%CV
G-Hb%	5.5	2.4	5.6	3.8	5.8	3.3	5.6	4.3
G-Hb%	13.0	2.5	13.2	3.7	14.2	3.6	13.9	4.8
G-Alb%-BCG	6.7	8.4	6.8	10.7	13.8	7.3	14.1	11.6
G-Alb%-ICG	5.9	3.3	6.0	4.2	12.9	3.7	13.0	4.4

Glyco-Gel™ Derived Non-diabetic Normal Range (N=100)						
Normal's Age Range	Glycated-Hb		BCG G-Alb.		ICS G-Alb	
	Mean	2SD	Mean	2SD	Mean	2SD
22-77 years	5.3	1.1	1.3	1.6	1.2	1.1

Well controlled diabetic patients were typically less than 8.0% G-Hb and 4.0% G-Alb(BCG). Assay results were found not to be significantly affected by room temperature, sample glucose < 4.0 g/L, or triglycerides < 8.0 g/L applied to the column. Thus, these assays were found to be sufficiently precise for clinical use in monitoring blood glucose control.

221 CRITICAL EVALUATION OF GLYCOSYLATED HEMOGLOBIN (HbA_1) MEASUREMENT IN CLINICAL PRACTICE, Silvia LENZI, Giovanna GIOVANNITTI, Tiziana SAMPIETRO, Ottavio GIAMPIETRO, Roberto MICCOLI, Giuseppe PENNO and RENZO NAVALESI (C.N.R. Clinical Physiology Institute, Catt.Malattie del Ricambio, Università,Pisa,ITALY)

To better assess the clinical usefulness of HbA_1 evaluation, we studied 234 diabetic patients (139 type I, 95 type II). They were followed in our Metabolic Center for 9-27 months period in which fasting plasma glucose (FPG), 24 hour urinary glucose (UG) and ketones (UK) were measured monthly, while HbA_1 was measured every three months. Regulation Index (RI) was calculated by a score system summing mean values of FPG, 24 hour UG and UK measured at least once in a month for three months prior to HbA_1 determination IR and HbA_1 positively correlated (r=0.50, p<0.01 in type I; r=0.71, p<0.01 in type II). However, it must be pointed out that in the "well-controlled" patient group (RI≤3) 44.8% of type I and 37.7% of type II subjects had HbA_1 > 9% (in our laboratory normal limits for HbA_1 are 6-8%). On the other hand, in the "poorly-controlled" diabetic group (RI > 3) only 5% had HbA_1 < 9, all of of them were type I presenting frequent and serious hypoglycemias.

We conclude that HbA_1 evaluation gives more information and so has more clinical relevance for those patients who show an "apparent" good control on the basis of only plasma and urinary glucose measurements.

222 A CATION-EXCHANGE HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ASSAY FOR HEMOGLOBIN A_{1C} AND HEMOGLOBIN SCREENING. Ching-Nan Ou, Pedro Carmona. (Dept. of Pathology, Texas Children's Hospital and Baylor College of Medicine, Houston, TX 77030).

A liquid chromatographic method using a poly (aspartic acid) cation exchange column is developed for the quantitation of hemoglobin A_{1C} and for the analysis of hemoglobins. The method uses a combined pH and ionic strength gradient elution system using the mobile phases as described by Ou et al. (J. Chromatogr. 266:197, 1983). The total run time is 30 min. Comparison of HPLC measurement of hemoglobin A_{1C} with ion exchange column chromatography (Isolab) yields the following results: y (HPLC method) = 0.96 x + 0.16, r = 0.949, n = 89 and Sy.x = 0.87. The day-to-day precision of the assay is less than 3%. The commonly encountered abnormal hemoglobins such as S.C.D.E.O.G.SG. can be easily identified without further confirmation by other methodology.

The high resolution of system and simplicity of the method plus the automation make the procedure useful for the accurate measurement of hemoglobin A_{1C} and screening of common hemoglobin disorders.

223 USE OF A KINETIC MODEL TO PREDICT CHANGES IN PERCENT HbAlc IN RESPONSE TO THERAPY, Richard E. Mullins and Garth E. Austin (Dept. Path. Lab. Med., Emory Univ. Sch. Med. and Atlanta VA Medical Center, Atlanta GA 30322 and 30033)

To extend the utility of glycated Hb measurements in assessing the response of diabetic patients to therapy, we determined an overall rate constant for the formation of glycated Hb and developed a kinetic model capable of predicting HbAlc levels. The differential rate equation for the reaction glucose + Hb $\underset{k_2}{\overset{k_1}{\rightleftharpoons}}$ aldimine $\xrightarrow{k_3}$ HbAlc, is $d(Alc)/dt = \frac{K_1K_3}{K_1+K_3}(glu)(Hb)$, where $k_1k_3/k_2+k_3 = \hat{k}$. Substituting values for k_1, k_2 and k_3, which were determined in vitro by Wykamp and Perdy (Clin. Chem. Acta, 125:341, 1982) yields a $\hat{k}$ of $8.8 \cdot 10^{-6}$ L/mmol·hr, which compares favorably with values of 7.5 and $7.8 \cdot 10^{-6}$ L/mmol·hr calculated empirically from patient data (Beach, J. Theor. Biol., 81:547, 1979; Mortensen and Volund, Diab. and Metabol., 10:18, 1984). Using a consensus value for $\hat{k}$ of $8.0 \cdot 10^{-6}$ L/mmol·hr and applying a correction factor for the loss of glycated Hb molecules from the available pool of nonglycated Hb, tables were constructed relating changes in blood glucose concentration to HbAlc percentage. For example, reductions of blood glucose from an initial steady state value of 20 mmol/L (23% HbAlc) to a final steady state value for glucose of 17, 14, 11, 8, or 5 mmol/L are calculated to reduce HbAlc values after 30 days to 20.7, 20.0, 19.4, 18.8 and 18.3 percent respectively, and after 60 days to 17.6, 16.5, 15.5, 14.2 and 13.3 percent. Failure to achieve the expected reductions in percent HbAlc would alert the clinician to the need to modify therapy or investigate patient compliance. More complicated situations have also been analyzed by this approach. The availability of quantitative information of this kind designed for a variety of clinical situations should greatly facilitate the use of HbAlc by the practicing physician in achieving diabetic control.

224 DYSHAEMOGLOBINS-, ESPECIALLY CARBOXYHAEMOGLOBIN,LEVELS IN HOSPITALIZED PATIENTS. A. Zwart and E.J. van Kampen. (Clin. Chem. Lab., Diakonessenhuis, Groningen, The Netherlands)

Since the determination of total haemoglobin concentration (C*Hb) in blood has been standardized as a routine- and reference HiCN-method (1,2), it became more and more evident that the dyshaemoglobins, i.e. carboxyhaemoglobin (HbCO), haemiglobin (Hi), and sulfhaemoglobin (SHb), have a tremendous negative influence on oxygen transport. In case of elevated dyshaemoglobin-values, most frequently HbCO, the percentage found must be multiplied with a factor 3 to 4 to obtain the percentage of C*Hb which *physiologically* does not function anymore (the so-called tetrameric effect). Simultaneous determination of dyshaemoglobins is carried out routinely in our laboratory with a spectrophotometric *multicomponent* analysis method (3). The method is very accurate and requires only two minutes time. Besides on special medical request, every patient is also checked in preoperative care for the possible presence of dyshaemoglobins. We obtained during a period of one year the following HbCO-distribution in 3022 surgery patients: normal HbCO (≤ 1.5%) = 65.0%; 1.5% < HbCO ≤ 5% = 26.5%; 5% < HbCO ≤ 10% = 8.2% and HbCO > 10% = 0.3%. This means that every 12th patient has an oxygen binding loss of about 25%, i.e. only 75% of C*Hb present, functions properly. The 35% of the patients, who have an abnormal HbCO-percentage, corresponds very well to the percentage smokers in The Netherlands. As blood-banks do not select their donors on smoking habits, until now, it is not surprising that about the same HbCO-distribution in blood-bags was found. Consequently this means that an appreciable portion of the bankblood is not as optimal for oxygen transport as the C*Hb of this blood suggests.

1. E.J. van Kampen and W.G. Zijlstra. Advances in Clinical Chemistry 8, 142-185 (1965). 2. E.J. van Kampen and W.G. Zijlstra. Advances in Clinical Chemistry 23, 200-253 (1984). 3. A. Zwart, Anneke Buursma, E.J. van Kampen and W.G. Zijlstra. Clin. Chem. 30, 373-379 (1984).

225 CORRELATION OF HEMOGLOBIN Alc LEVELS WITH DIABETIC HISTORY USING SAMPLES FROM RANDOM HOSPITAL PATIENTS, C. Cecil Cupperr, Cathy Mrozek, Vicki Bergman and Diane Varmecky (Dept. Pathol., Lee Hospital, Johnstown, PA 15901)

The purpose of this study was to: (a) investigate our ability to identify undiagnosed diabetics, (b) evaluate the levels of HbAlc in patients with a diabetic history, and (c) confirm the