Letters to the Editor

A Simple Method for Routine Measurement of Glycosylated Haemoglobin

Dear Sir,

Ion-exchange chromatography is commonly used for determining haemoglobin A₁ (HbA₁) [1–3]. Micro-scale assays are attractive [3], but present practical difficulties such as adjustment of the phosphate buffer to the correct pH. This step can be avoided by precise weighing of the phosphates on an analytical balance. We weigh out 3.2746 g of Na₂HPO₄ precisely, while keeping the rest of the buffer components as originally described [3]. In our experience this modification leads to a stable and reproducible pH of buffer A; pH 6.740 with redistilled water and pH 6.725–6.735 with monodistilled water. Routinely we use monodistilled water and equilibrate the resins to the pH value of buffer A, i.e. to 6.730. The ionic strength of the buffer prepared in this way is always 0.11, i.e. in the best range for the ion-exchange technique we use. We have used not only Bio-Rex 70 (200–400 mesh; Bio-Rad Laboratories, Richmond, California, USA) but have also compared it with Amberlite CG 50 (100–200 mesh; Fluka, Buchs, Switzerland) and found that using the latter shortens the chromatographic procedure from 2 h to 40 min. We found that performing the chromatography at a constant temperature of 22 °C in a water bath increased the reproducibility of the results. Amberlite CG 50 columns showed an intra-assay coefficient of variation of 1.8% and an interassay coefficient of variation of 3.9%, compared with 3% and 4% respectively as shown by Welch and Boucher [3].

We chromatographed 15 haemolysates simultaneously on Amberlite CG 50, on Bio-Rex 70 columns and with electro-focussing on an LKB: 1804-103 PAG Plate (pH 5.5–8.5) (LKB, Bromma, Sweden).

Mean results for HbA₁ with Amberlite CG 50 were 13.35% and with Bio-Rex 70 13.48% ($p > 0.10$, $r = 0.9966$). The mean HbA_{1c} with isoelectric focussing was 10.46% and the correlation coefficient between HbA_{1c} determined by isoelectric focussing and HbA₁ by chromatography were 0.8857 for Amberlite CG 50 and 0.8664 for Bio-Rex 70.

Our method has the convenience of repeated re-use of the same resin. After each assay, the resins are regenerated. They are washed once with an excess of 0.5 N NaOH to a pH > 10 and rinsed several times with distilled water to a pH of approximately 9. This is followed by washing in 0.5 N HCl to a pH of approximately 1 and then in distilled water to approximately pH 4. The resin is then washed twice with buffer A (five volumes). To reach the pH of buffer A (6.730), it is necessary to adjust the titrate of the buffer six times with 0.5 N NaOH to a pH of approximately 6.740–6.750. (The first washing with buffer A results in a pH of the resin below 6.700, after the second and third washes the pH reaches 6.710, and after the last wash it is approximately 6.730.) The resin has then to be washed three to four times with buffer A to allow the eluates to reach the ionic strength of buffer A. The equilibration of the resin is controlled with a pHM64-meter (Radiometer, Copenhagen, Denmark). The resin is then ready for use.

Thirty healthy subjects examined with our method (mean age 36 years) gave $7.78 \pm 0.67\%$ (mean $\pm$ SD); 28 well controlled and 32 decompensated diabetic patients gave $10.46 \pm 1.79\%$ and $15.21 \pm 2.71\%$, respectively.

In our experience these modifications make a convenient and inexpensive method for routine measurement of HbA₁ in diabetic clinics, thus providing a reliable measure of diabetic control.

Yours sincerely,

M. Nikolov and L. Damianova

References

1. Trivelli LA, Ranney HM, Lai HT (1971) Hemoglobin components in patients with diabetes mellitus. *N Engl J Med* 284: 353–357

2. Kynoch PAM, Lehman H (1977) Rapid estimation (2½ h) of glycosylated haemoglobins for routine purposes. *Lancet* 1: 16–18
3. Welch SG, Boucher BJ (1978) A rapid micro-scale method for the measurement of haemoglobin A_{1(a+b+c)}. *Diabetologia* 14: 209–211

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Absence of Rapid Glycosylation of Rat Haemoglobin

Dear Sir,

Recently Vialettes et al. [1] analyzed in this journal the kinetics of fast (glycosylated) haemoglobin (GHb) in Wistar rats by means of an ion-exchange chromatography and iso-electric focussing. The colorimetric assay of GHb is especially suitable for the study of haemoglobin glycosylation in rats [2] because it overcomes the problem of rat haemoglobin heterogeneity [3] and is not influenced by the labile aldime form of GHb [4].

The two-to threefold elevation of GHb levels measured by our modification of the colorimetric method [5] in insulin-treated alloxan diabetic rats (blood glucose before insulin treatment: 18–39 mmol/l) was in good agreement with that found by Vialettes et al. and by Blanc et al. [1, 6].

However, when healthy Wistar rats (180–220 g) were made hyperglycaemic after an overnight fast by intraperitoneal injection of glucose (8 g/kg body weight, in the form of 20% solution of glucose), we did not observe any change in GHb concentration during and after the hyperglycaemic period (Table 1). Our finding indirectly confirms the opinion that the rapid glycosylation of haemoglobin is an artefact due to its labile aldime form [7].

Our experiment does not touch the problem of the fast deglycosylation of haemoglobin. When valuating such events, one has to consider the fact that the oldest red blood cells carrying the highest load of GHb are eliminated shortly after restoration of euglycaemia [8].

Yours sincerely,

O. Racz and M. Riemerova

Table 1. Glycaemia and GHb during and after short-term hyperglycaemia in eight healthy Wistar rats

Time after injection of glucose (h)	Glycaemia (mmol/l)	GHb (mmol 5-HMF ^a /mol Hb)
0	4.7 $\pm$ 0.3	20.1 $\pm$ 2.6
½	53.3 $\pm$ 6.2	19.9 $\pm$ 2.8
1	47.5 $\pm$ 7.1	19.7 $\pm$ 2.8
2	39.3 $\pm$ 9.2	20.1 $\pm$ 3.0
4	18.6 $\pm$ 4.8	20.4 $\pm$ 2.4
8	4.6 $\pm$ 0.4	20.2 $\pm$ 2.1
24	4.4 $\pm$ 0.4	20.3 $\pm$ 2.2

Results are expressed as mean $\pm$ SD.

^a 5-hydroxymethylfurfural

References

1. Vialettes B, Vovan L, Simon MC, Lassmann W, Altomare E, Vague P (1982) Kinetics of fast haemoglobin in diabetic rats. *Diabetologia* 22: 264–268
2. Flückiger R, Winterhalter KH (1976) In vitro synthesis of hemoglobin A_{1c}. *FEBS Lett* 71: 356–360
3. Garrick LM, Sharma VS, McDonald MJ, Ranney HM (1975) Rat haemoglobin heterogeneity. *Biochem J* 149: 245–258
4. Bunn HF (1981) Evaluation of glycosylated hemoglobin in diabetic patients. *Diabetes* 30: 613–617
5. Riemerová M, Rác O, Varga J (1982) Glycosylation of hemoglobin in rats. *Bratislavské Lekárske Listy* 79 (in press) (Slovak with English abstract)
6. Blanc MH, Rhie FH, Dunn PJ, Soeldner JS (1981) The determination of glycosylated hemoglobins in rats using high pressure liquid chromatography. *Metabolism* 30: 317–322
7. Sevendsen PA, Christiansen JS, Soegaard U, Welinder BS, Nerup J (1981) Rapid changes in chromatographically determined haemoglobin A_{1c} induced by short-term changes in glucose concentration. *Diabetologia* 19: 130–136
8. Beach KW (1979) A theoretical model to predict the behaviour of glycosylated hemoglobin levels. *J Theor Biol* 81: 547–561

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Red Blood Cell Volume and Glycaemic Control in Diabetes

Dear Sir,

The occurrence of an increased mean red cell volume (MCV) in diabetes, not correlated with glycosylated haemoglobin (HbA_{1c}), has been reported by Davidson et al. [1]. This finding has been recently

Table 1. Red blood cell volume and indices of diabetic control

	Control Subjects (n = 50)	Diabetic patients (n = 50)
Age (years)	40 ± 7.8	41 ± 7.0
Sex (M:F)	25:25	25:25
Blood glucose (mmol/l)	4.95 ± 0.45	9.85 ± 0.74
Stable HbA _{1c} (%)	6.23 ± 0.68	10.18 ± 1.70
Glycosylated serum proteins (HMF ^a nmol/mg protein)	0.64 ± 0.26	0.95 ± 0.42
Mean corpuscular volume (fl)	87.87 ± 3.54	90 ± 5.87

Data are expressed as mean ± SD; ^a HMF = Hydroxymethylfurfural

questioned by Beautyman [2], who observed that severe hyperglycaemia may cause inaccuracy in automated measurement of corpuscular indices [3].

We investigated MCV, blood glucose, stable HbA_{1c} and glycosylated serum proteins (which measure long- and short-term control respectively [4]) in 50 healthy subjects and in 50 diabetic patients matched for age and sex (Table 1). An increased MCV in the diabetic patients (90 ± 5.87 versus 87.87 ± 3.54 fl, mean ± SD; *p* < 0.05) and a linear correlation between MCV and serum glycosylated proteins (*r* = 0.42, *p* < 0.01) were observed. The lack of correlation between MCV and stable HbA_{1c} and the good correlation with glycosylated proteins suggest that short-term metabolic control may influence MCV in diabetes.

Moreover, as the methods for measurement of stable HbA_{1c} and glycosylated proteins were not affected by free serum glucose (both methods, by dialyzing samples, remove labile glucose adducts [5, 6], our data provide further evidence that changes in glycaemic control in diabetes may lead to haematological alterations, such as polycythaemia [7] and increased reticulocyte counts [8].

Yours sincerely,

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References

1. Davidson RJL, Evan-Wong LA, Stowers JM (1981) The mean red cell volume in diabetes mellitus. *Diabetologia* 20: 583–584
2. Beautyman W (1982) Red cell volume in diabetes. *Diabetologia* 22: 220 (Letter)
3. Strauchen AJ, Alston W, Anderson J, Gustafson Z, Fajardo LF (1981) Inaccuracy in automated measurement of hematocrit and corpuscular indices in the presence of severe hyperglycaemia. *Blood* 57: 1065–1067
4. Kennedy AL, Merimee TJ (1981) Glycosylated serum proteins and haemoglobin A_{1c} levels to measure control of glycaemia. *Ann Int Med* 96: 56–58
5. Compagnucci P, Cartechini MG, Bolli G, De Feo P, Santeusano F, Brunetti P (1981) The importance of determining irreversibly glycosylated haemoglobin in diabetics. *Diabetes* 30: 607–612
6. Kennedy AL, Mehl TD, Merimee TJ (1980) Non-enzymatically glycosylated serum protein: spurious elevation due to free glucose in serum. *Diabetes* 29: 413–415
7. Graham JJ, Ryall RG, Wise PH (1980) Glycosylated haemoglobin and relative polycythaemia in diabetes mellitus. *Diabetologia* 18: 205–207
8. Ceriello A, Dello Russo P, Sgambato S, Giugliano D (1982) Glycosylated haemoglobin and reticulocyte count in diabetes. *Diabetologia* 22: 223 (Letter)

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Book Reviews

J. C. Brown. Gastric Inhibitory Polypeptide (Monographs on Endocrinology). Berlin, Heidelberg, New York: Springer 1982. 32 figs, pp 88, hardback DM 68.00/US \$ 30.20. ISBN: 3-540-11271-5

J. C. Brown, the discoverer of gastric inhibitory polypeptide presents a monograph on this hormone. This book tells in a concise form the story of the analysis of an observation leading to the discovery of a new

hormone. The first section covers the problem of enterogastrone and incretin, two physiological principles, which, at least in part, are believed to be related to gastric inhibitory polypeptide (GIP). In section two, the chemistry of GIP is described together with the recent correction of the amino acid sequence. Modern separation and isolation techniques are presented and details on the biological activity of synthetic fragments of GIP are given. Chapter three describes the physio-