Review article
Glycosylated haemoglobin: measurement and clinical use

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SUMMARY The discovery, biochemistry, laboratory determination, and clinical application of glycosylated haemoglobins are reviewed. Sources of error are discussed in detail. No single assay method is suitable for all purposes, and in the forseeable future generally acceptable standards and reference ranges are unlikely to be agreed. Each laboratory must establish its own. Nevertheless, the development of glycosylated haemoglobin assays is an important advance. They offer the best available means of assessing diabetic control.

Following widespread recognition that sustained hyperglycaemia is an important determinant of the long term complications,[1][2] there has been renewed interest in monitoring control of diabetes.[3] The discovery of glycosylated haemoglobin has therefore been timely; measurement of it should allow more objective assessment of control than has previously been possible. There are, however, technical problems to be solved before this promise can be fully realised.

Background

The story begins in the 1950s with studies of the electrophoretic and chromatographic heterogeneity of haemoglobin in non-diabetic subjects.[4]–[6] Several varieties of haemoglobin were found in low concentrations and were proved not to be artefacts. In 1968 Bookchin and Gallop[7] subsequently reported that the largest of these minor fractions, designated Hb A_{1c}, had a hexose moiety linked to the N-terminus of the β-globin chain.

Working independently, Rahbar[8] reported that an abnormal fast moving haemoglobin fraction was present in just two of 1200 patients tested in Tehran; both had diabetes. Huisman and Dozy[9] had previously attributed an increase in fast moving haemoglobin in four diabetic patients to the tolbutamide they were being treated with. Further

studies by Rahbar and colleagues[10] showed that this haemoglobin variant found in abnormally high concentrations in diabetes was identical to the Hb A_{1c} originally identified by Allen *et al*.[6]

In a study of identical twins concordant and discordant for diabetes, Tattersall *et al*[11] showed that the abnormal proportion of fast haemoglobin found in diabetes was an acquired manifestation of metabolic abnormality and not, as had been suggested, an inherited marker for diabetes.

The final and crucial observation which led to the use of assays of abnormal haemoglobins as a method of assessing diabetic control was the demonstration by Koenig *et al* that Hb A_{1c} concentration was proportional to fasting blood glucose concentration and glucose tolerance.[12] Furthermore, they showed that Hb A_{1c} concentrations fell when diabetic control was improved by treatment.[13]

Biochemistry

STRUCTURE

We now know that there are many different "glycosylated haemoglobins." The structure and nomenclature of those best known are shown in the Table.

All the glycosylated haemoglobins have a carbohydrate moiety (glucose or a derivative) attached to one of the globin chains. Carbohydrate may be attached to the N-terminal amino acid residue (valine) of the α or β globin chains, or to lysine residues within each chain.[14] Linkage to the N-terminus of the β chains is of most practical importance since

Structure and nomenclature of haemoglobin variants

Name	Globin subunit composition	Proportion found in	
		Non-diabetics	Diabetics
Non-glycosylated ("native") haemoglobins			
Hb A (Hb A_0)	$\alpha_2\beta_2$	>80%	60–80%
Hb A_2	$\alpha_2\delta_2$	2%	2%
Hb F	$\alpha_2\gamma_s$	<1%	<1%
Glycosylated derivatives not specifically named	$(\alpha$-Val-1-deoxyfructose$)_2\beta_2$ $(\alpha$-(Lys-glucose$)_n)_2\beta_2$ $(\alpha_2(\beta$-(Lys-glucose$)_n)_2$	?8–10% (ref 14)	Unknown, but increased
Hb A_{1a1} Hb A_{1a2} often named (and Hb A_{1b} assayed) collectively Hb A_{1c} as Hb A_1	$\alpha_2(\beta$-Val-fructose diphosphate$)_2$ $\alpha_2(\beta$-Val-glucose-6-phosphate$)_2$?(ref 96) $\alpha_2(\beta$-Val-1-deoxyfructose$)_2$	<1% <1% <1% 4%	<1% <1% <1% Up to 20%

this gives rise to the altered physical properties that are exploited in assays. In particular, chromatographic mobility is increased—hence the name "fast haemoglobins" by which these compounds are commonly known. Adducts elsewhere on the haemoglobin molecule have less effect on its properties.

FORMATION

Glycosylated haemoglobins are the result of simple chemical reactions between haemoglobin and sugars after synthesis of haemoglobin is complete—that is, post-translational modifications.
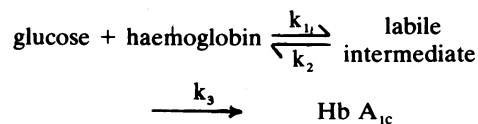
Because of the practical importance of Hb A_{1c} its synthesis has been studied extensively.[15][16] The reaction proceeds in two stages (Fig. 1); enzymes are not involved.

1 Glucose combines with the α amino group of the valine residue at the N-terminus of β globin chains to form an aldimine compound (Schiff base). This reaction is reversible, and dissociation to native haemoglobin and glucose occurs readily.

2 Internal rearrangement of the aldimine intermediate by the Amadori reaction yields a stable ketoamine derivative.

REACTION KINETICS

Early workers confirmed that glycosylation begins during erythropoiesis and continues slowly throughout the life of haemoglobin in the circulation; concentrations reached in the red cells of diabetic subjects are consistent with their known lifespan of about 120 days.[16]

The formation of Hb A_{1c} is conveniently expressed by a simple equation:

$$\text{glucose} + \text{haemoglobin} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \begin{array}{l}\text{labile}\\\text{intermediate}\end{array}$$

$$\xrightarrow{k_3} \text{Hb } A_{1c}$$

in which k_1, k_2, and k_3, are the reaction rate constants. Beach[17] showed that the concentrations of Hb A_{1c} found in experimental situations where the glucose concentration is known can be predicted by such a first order reaction model, as can the apparent "saturation" effect at higher prevailing glucose concentrations, first pointed out by Graf *et al.*[18] In glucose clamp experiments Svendsen *et al*[19] have shown that the amount of stable Hb A_{1c} formed in 24 h is 0·006% of total haemoglobin per mmol of glucose. As will emerge later, the reaction rate constants are of crucial importance.

Clinical studies

Following the original demonstrations[12][13] that glycosylated haemoglobin concentrations reflect diabetic control, many workers have tried to define the relation more precisely. Two difficulties have complicated the task.

1 Previous indices of diabetic control were at best imprecise. Thus there has been no simple, reliable, and widely accepted measure by which to judge the new tests. No one has access to continuous and accurate records of blood glucose over long periods—the ultimate standard.

2 Unstable intermediates are now known to interfere with assay results. The early belief that glycosylated haemoglobin results would not reflect short term fluctuations in blood glucose turned out to be partly incorrect.

Despite these difficulties, many cross sectional and some longitudinal studies have been completed.[20] Glycosylated haemoglobin concentrations have been shown to be related to: physicians' ratings of diabetic control,[21] fasting blood glucose concentrations,[21][22] postprandial blood glucose concentrations,[22] mean blood glucose concentrations during monitoring in hospital,[21] peak blood glucose concentrations during monitoring in hospital,[21] home
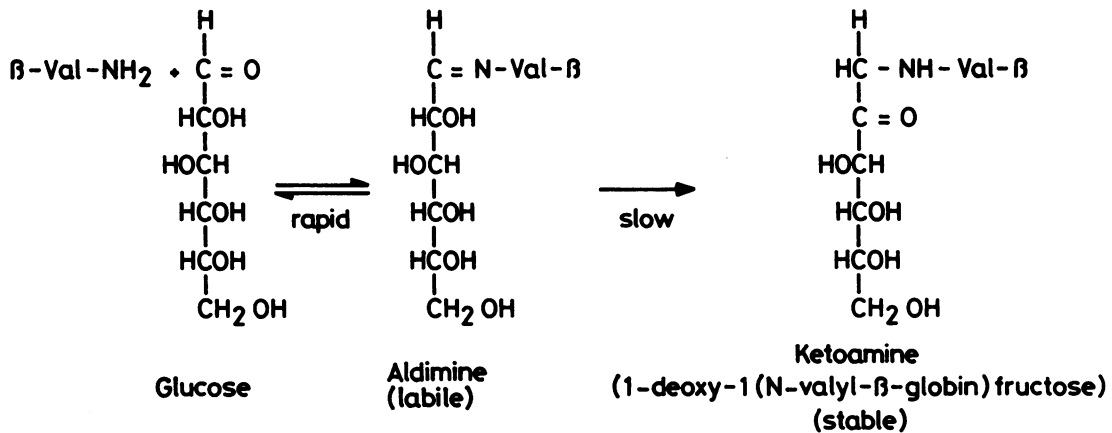
$$\beta\text{-Val-NH}_2 + \begin{matrix} H \\ | \\ C=O \\ | \\ HCOH \\ | \\ HOCH \\ | \\ HCOH \\ | \\ HCOH \\ | \\ CH_2OH \end{matrix} \quad \underset{rapid}{\rightleftharpoons} \quad \begin{matrix} H \\ | \\ C=N\text{-Val-}\beta \\ | \\ HCOH \\ | \\ HOCH \\ | \\ HCOH \\ | \\ HCOH \\ | \\ CH_2OH \end{matrix} \quad \xrightarrow{slow} \quad \begin{matrix} H \\ | \\ HC-NH\text{-Val-}\beta \\ | \\ C=O \\ | \\ HOCH \\ | \\ HCOH \\ | \\ HCOH \\ | \\ CH_2OH \end{matrix}$$

Glucose   Aldimine (labile)   Ketoamine (1-deoxy-1 (N-valyl-β-globin) fructose) (stable)

Fig. 1 *Reactions leading to the formation of haemoglobin A_{1c}.*

urine test results,[22] 24h glycosuria,[22 23] and home monitoring of blood glucose concentrations.[24]

Measurement of Hb A_{1c} has turned out to be particularly useful. Not only is it more plentiful than the other easily separable glycosylated haemoglobins, but its concentration does vary widely in relation to diabetic control. Changes of Hb A_{1a} and Hb A_{1b} are neither so large nor so easily measured.

TIME COURSE OF CHANGES
When glucose concentrations are stable (whether normal or abnormal) glycosylated haemoglobin concentrations reflect average blood glucose values over the preceding weeks, the mean value in the red cell population being that contained by those cells of an age equal to half their average life span—namely, 50–60 days.

Early investigations indicated a lag phase between changes in diabetic control and the resulting changes in Hb A_{1c}. Koenig *et al*[13] showed that improved control was followed by a fall in Hb A_{1c} only after 3–4 weeks. Such a delay is expected on theoretical grounds. Once formed, stable Hb A_{1c} (but not the unstable Schiff base intermediate) remains in red cells until they are removed from the circulation. A significant reduction of overall glycosylated haemoglobin concentration through appearance of newly formed cells containing unmodified haemoglobin must therefore await the death and removal of older cells. By contrast, a relatively short period of poor control can rapidly and irreversibly increase glycosylation in all generations of red cells in the circulation. In other words, glycosylation is much more sensitive to sin than repentance. This theoretical prediction has been confirmed by three studies in which treatment was deliberately suspended.[25–27].

HYPOGLYCAEMIA
A period of good control will not undo the glycosylation of earlier times. Therefore, a short period of hypoglycaemia before measurement of Hb A_{1c} will not alter the result. Reduced glycosylation during repeated episodes of prolonged hypoglycaemia may, however, mask the effect of intervening periods of hyperglycaemia, with the result that Hb A_{1c} concentrations may be near normal or even low. These results may be falsely interpreted as an indication of sustained normoglycaemia. Regular nocturnal hypoglycaemia, which when looked for carefully is common among insulin treated patients, is particularly likely to cause such misleading results.[28 29]

FAST GLYCOSYLATION
In a study of diabetic children who were closely monitored by regular blood and urine testing, Dunn *et al*[30] showed that while Hb A_1 values correlated with various measures of glycaemia over several preceding weeks, they were most closely related to blood glucose during the week immediately before blood was taken for Hb A determination. Svendsen *et al*[31] suggested that chromatographically determined Hb A_{1c}, or part of it, was in fact formed faster than had been believed previously. After considerable dispute about fast glycosylation Ditzel proposed,[32] and later confirmed,[33] that the reversible short term intermediate Schiff base reacts in many assays. Since k_1 is greater than k_2 and k_3 in the equation (see above) a short period of hyperglycaemia yields an acute excess of Schiff base that will cause an unrepresentative increase in total glycosylated haemoglobin. The solutions to this problem are discussed later.

Assay methods

For almost 20 years after the discovery of glycosylated haemoglobins chromatography was the only available method of assay. In 1971 Trivelli *et al*[34] presented results using a method essentially based on the large column technique described by Allen *et al* in 1958.[6] Since the mid seventies various less elaborate techniques have been devised and many are now available for routine laboratory use.

CATION EXCHANGE CHROMATOGRAPHY

Methods using microcolumns

Kynoch and Lehmann[35] described a column method that allowed estimation of glycosylated haemoglobin in two and a half hours; the DEAE-cellulose method of Huisman and Dozy[9] required several days for complete ·separation and Trivelli's technique[34] took 24 h. Kynoch and Lehmann used Bio-rex 70 cation exchange resin packed into 20 ml syringe barrels. Haemolysed red cells were placed on to the resin and then two buffer solutions were added in succession. The first eluted glycosylated haemoglobin and the second unmodified haemoglobin, the concentrations of each being measured by spectrophotometry.

Subsequently, numerous authors have described methods that have been further scaled down, allowing assays to be performed quickly and in large numbers. In addition, many disposable prepacked columns, supplied with suitable buffers, have been marketed.[36] In most cases they measure total haemoglobin A_1, and also the intermediate Schiff base, rather than Hb A_{1c} alone. However, Maquart *et al*[37] described a modification of the basic technique to allow assay of Hb A_{1c} specifically with microcolumns, and there are new kits under evaluation[38] that separate Hb A_{1c} from Hb A_{1a} and Hb A_{1b}.

The disadvantage of microcolumn methods is that they are sensitive to slight variations in conditions, especially to the ionic strength and pH of the eluting buffers, the rate of elution, and temperature.[39 40] As pointed out by Dix *et al*,[41] and subsequently confirmed by others, variations in ambient temperature can lead to changes in measured Hb A_1 concentration of the order of 10% per 2°C. Several companies now issue temperature correction nomograms, such as the one published by Hankins,[42] but these cannot compensate for temperature fluctuations *during* assay. Temperature controlled water jackets are available to hold the resin columns, but we have found them cumbersome and difficult to maintain at the correct temperature.

Batch chromatography[43]

In this method the resin and elution buffer are mixed to form a slurry, which is then agitated with haemolysate. Unmodified haemoglobin attaches to the resin, while glycosylated haemoglobin remains in the supernatant and can be measured spectrophotometrically after separation. Experience with the method is limited, but it is sensitive to changes in temperature in the same way as more conventional column methods.

AFFINITY CHROMATOGRAPHY[44 45]

Microcolumns packed with phenylboronic acid affinity gel offer a promising new approach. The diol moieties in glycosylated haemoglobins are bound selectively, allowing separation from unmodified haemoglobin. The technique seems to be less sensitive to changes in temperature and pH and the columns can be regenerated easily. It is particularly suitable for use in the study of diabetes in laboratory animals with heterogeneous haemoglobins which complicate assay by other methods.[46]

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY[47]

Not surprisingly, more sophisticated methods offer greater precision and allow separation of the various components of Hb A_1, but are more labour intensive and costly in equipment, at least initially.

COLORIMETRY

When heated with oxalic acid, ketoamine linked hexoses are hydrolysed to 5-hydroxymethylfurfuraldehyde (HMF). The addition of 2-thiobarbituric acid yields a coloured product which can be estimated photometrically. This reaction, originally reported by Fluckiger and Winterhalter,[48] showed initial promise as a means of estimating glycosylated haemoglobin, and standardised procedures have been published.[49−52] Not only the hexose groups on the terminal valine residues of globin chains, but also glycosylated lysine residues within the chains react to yield HMF. Aldimines do not react, however, so that interference by the short term intermediates is not a problem. Furthermore, as a chemical method there is greater scope for inclusion of standards. In practice, however, the technique has not found general favour; it is tedious and time consuming and calls for carefully controlled reaction conditions.

ISOELECTRIC FOCUSING[53 54]

Because of the high resolution which can be achieved isoelectric focusing is valuable as a research method. Commercial equipment is available and is particularly useful for identifying variant

haemoglobins which may be eluted together with Hb A$_1$ in microcolumn methods (see below). Another advantage is that stable Hb A$_{1c}$ is readily separated from the intermediate Schiff base.[55]

RADIOIMMUNOASSAY
There has been one report[56] that a specific antiserum developed in sheep against human Hb A$_{1c}$ could be used as the basis for a quantitative assay. The method has not yet been developed for general use.

SPECTROPHOTOMETRIC ASSAY[57]
Phytic acid binds to haemoglobin, altering its optical absorption properties. Glycosylated haemoglobin does not bind phytic acid. Thus the percentage of glycosylated haemoglobin in a blood sample is inversely proportional to the difference in absorption before and after addition of phytic acid. The method is quick, simple, and not temperature dependent, but it does depend on the use of a precision spectrophotometer with high resolution.

ELECTROPHORESIS/ELECTROENDOSMOSIS
Glycosylation of haemoglobin changes its isoelectric point by only 0·01 pH unit.[58] It is therefore not surprising that conventional electrophoretic techniques have not proved suitable for assay of Hb A$_1$. Menard *et al*,[59] however, described a method using agar gel plates on which separation of Hb A$_1$ is achieved by electroendosmosis. Now commercially available, the technique is relatively simple, quick, and not temperature dependent, but reading of the plates requires careful setting of the scanning densitometer to the correct baseline.

A further development has been the use of plastic supported cellulose acetate membranes and the principle of mobile affinity electrophoresis.[60] Glycosylation blocks the affinity of haemoglobin for the sulphate groups of dextran sulphate in electrophoresis buffers and renders Hb A$_1$ relatively immobile. Protein staining of the plates improves the reliability of scanning. The method is relatively unaffected by temperature, pH, and buffer strength and promises to be economical.

Most laboratories offering routine glycosylated haemoglobin assays use either microcolumns or the endosmosis technique. Colorimetric, spectrophotometric, or batch chromatographic methods are practical but less popular alternatives. Boucher *et al* have recently compared the different methods.[61] They did not recommend any one, pointing out the advantages and drawbacks of each. They drew attention to the lack of available standards and to the need for doctors to be aware of changes in methodology that might influence results.
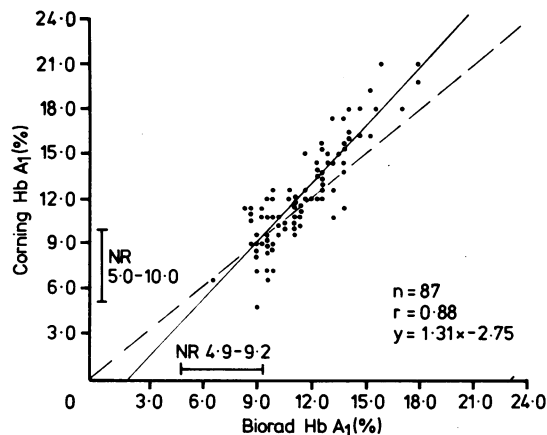

Fig. 2 *Comparison of duplicate results obtained by Corning (electroendosmosis) and Biorad (ion exchange chromatography) assays. NR = normal range (mean ± 2SD in non-diabetics). Solid line indicates regression line, broken line indicates 45° slope (axes are asymmetrical).*

It should be remembered that the principles of the different methods vary so widely that even the population of glycosylated haemoglobins measured may be different. A reference range should therefore be established for each method. There may not even be a linear relation between results obtained using different methods. A comparison of results of duplicate samples assayed in our laboratories by microcolumn chromatography (Bio-Rad) and endosmosis (Corning) is shown in Fig. 2. (The samples were not treated to remove aldimine intermediates; these are included with Hb A$_1$ by both methods.)

For clinical purposes non-specific assay of total Hb A$_1$ is technically easier and gives adequate results, but for research purposes optimal sensitivity is obtained from those assays that specifically measure Hb A$_{1c}$.

Sources of error
HANDLING OF SPECIMENS BEFORE ASSAY
With the exception of the colorimetric technique, all methods in common use measure the labile aldimine intermediate along with Hb A$_1$. A short period of hyperglycaemia before blood is taken leads to an acute increase in the formation of aldimine which may increase the concentration of glycosylated haemoglobin by 10–20%—for example, from 9% to 11% of total haemoglobin[62]—thus reducing the reliability of the test as a measure of *long term* diabetic control. Blood samples should therefore be treated to remove the aldimine before assay.

Ditzel *et al*[33] recommend formal dialysis, but others have shown that unstable intermediates dissociate during simple incubation of red cells in

isotonic saline. There has been considerable debate over the optimum incubation temperature, dilution, and length of time necessary. Svendsen *et al*[63] recommend 37°C, though Paisey *et al*[64] express concern that haemoglobin may be denatured at this temperature and Shenouda *et al*[65] agree that 20-fold dilution overnight in isotonic saline at room temperature is effective; this is certainly a practical method. Since glycosylation will continue in vitro if blood glucose is high when blood is sampled, incubation in saline should be started as soon as possible. After treatment the sample should be stored at 4°C until assayed, or if there is to be a long delay, as a haemolysate at −70°C.[66 67]

Nathan *et al*[68] have suggested semicarbazide and aniline treatment of samples at pH 5 to eliminate the aldimine intermediate, but these chemicals are toxic. Hydrazine has also been suggested and Bisse *et al*[69] and Bannon[70] report that simple incubation at pH 5 without additives is sufficient, but these methods have not yet been widely applied. Care must be taken to avoid denaturation.

The need to eliminate labile intermediates prevents reliable immediate measurement of Hb A$_1$ at the time of clinic visits, a practice suggested by Saunders *et al*.[71] Accuracy should not be sacrificed for speed.

TECHNICAL PROBLEMS
Under carefully regulated conditions, within batch and between batch coefficients of variation may be less than 3%, or even as low as 1%; in routine practice, however, coefficients of variation of 5% or even 7% are to be expected. Most commercial kits now include low and high lyophilised haemoglobin controls, but unless these are included in duplicate (or ideally in triplicate) it is unwise to use them to correct results. Such rigorous quality control is difficult when using electrophoretic methods, since most commercially available plates or membranes have room for only eight samples, although strictly speaking standards should be included on every plate as there is undoubtedly variation between them (especially between plates from different batches). Also, as previously mentioned, unless the densitometer is set to the correct baseline, there may be bias in all the results from any one plate. The effect of temperature on microcolumn assays has already been discussed—again, although manufacturers do provide standards, their use to correct results requires meticulous care.

BIOLOGICAL AND PHARMACOLOGICAL VARIABLES
Any cause of shortened red cell survival will reduce exposure to glucose, with a consequent decrease in

Hb A$_1$ values. Bunn *et al*[16] showed that non-diabetic patients with haemolytic anaemia had abnormally low Hb A$_{1c}$ concentrations (1–3%) and Kesson *et al*[72] described a diabetic patient with chronic hyperglycaemia and a Hb A$_1$ value of only 4%, owing to haemolysis induced by dapsone. Saddi *et al*[73] showed that Hb A$_{1c}$ values in patients with haemochromatosis fell after frequent venesections.

Brooks *et al*[74] noted that iron deficiency was associated with increased Hb A$_1$ concentrations in non-diabetic patients. The mechanism is not clear; Hb A$_1$ returns to normal after iron treatment.

Haemoglobin variants may cause spuriously high results with some assay methods. Haemoglobin F and haemoglobin H elute from microcolumns together with haemoglobin A$_1$.[75] A woman attending our clinic was found by column chromatography to have an apparent haemoglobin A$_1$ of 26·7% despite blood glucose concentrations consistently below 10 mmol/l. Haemoglobin electrophoresis confirmed that she had abnormal (we presume inherited) persistence of haemoglobin F. This example illustrates the potential pitfalls of total reliance on Hb A$_1$ values alone.
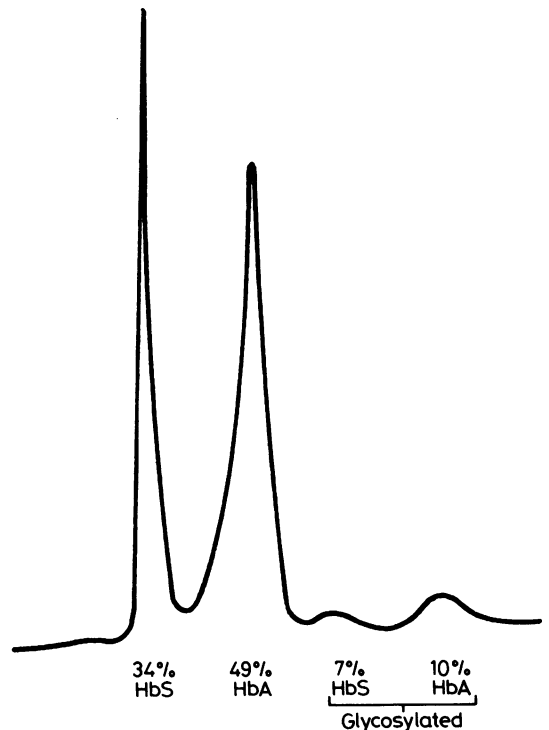

Fig. 3 *Separation of haemoglobin variants and their glycosylated derivatives by electroendosmosis in a patient with a high concentration of haemoglobin S.*

By contrast, Sosenko *et al*[76] showed that chromatographic estimation of glycosylated haemoglobin in patients with haemoglobins S, C, or D gives falsely low results. Glycosylation of these variants is comparable with that of haemoglobin A, but their glycosylated derivatives do not elute with haemoglobin A_1 from columns. High resolution methods such as isoelectric focusing will reveal the various glycosylated haemoglobins, and colorimetric techniques will give correct results; both Hb A_1 and the glycosylated haemoglobin variant yield HMF after hydrolysis. Fig. 3 shows that electroendosmosis also reveals the truth in these circumstances.

The situation in uraemia is complex. Dandona *et al*[77] reported low levels of Hb A_1, consistent with the known shortening of red cell life span. Schernthaner *et al*[78] described increased levels of Hb A_1, however, and showed a correlation with plasma creatinine. Fluckiger *et al*[79] have subsequently shown that the apparent increase of Hb A_1 is largely caused by haemoglobin carbamylation resulting from a condensation of urea derived cyanate with the N-terminal amino group of globin chains. This product elutes with Hb A_1 from columns, but is not detected by colorimetric assays specific for glycosylation.

Ingestion of aspirin in high doses can cause a spurious increase in glycosylated haemoglobin measured by chromatography or electroendosmosis. Colorimetric techniques and isolectric focusing, however, distinguish glycosylated fractions from the acetylated derivative of haemoglobin resulting from aspirin.[80]

Finally, a word of caution. Stevens *et al*[81] have suggested that unusual haemoglobin adducts may form in people abusing alcohol. They have shown that acetaldehyde reacts with amino acid residues in globin chains producing higher concentrations of minor haemoglobin variants in heavy drinkers than in controls, although this finding has not yet been confirmed.

Use in clinical practice

Having reviewed the theory, we must now ask what is the value of glycosylated haemoglobin measurement? Like any other assay, it will not be useful unless the result leads to action, ideally action that will improve patient care.

The promise of glycosylated haemoglobin is that it will be a reliable yardstick of diabetic control that is simple, objective, and representative of average blood glucose concentrations over several weeks. Other advantages are that it is independent of patient compliance and, if steps are taken to remove aldimine, not affected by short term fluctuation.

Previously there has not been such an index; in past years physicians caring for diabetic patients have had little hard information to go by. Accurate assessment of diabetic control has not been possible until now.

Freedom from the dramatic short term complications of diabetes—namely, ketoacidosis and hypoglycaemia—is no guarantee of good control. Many patients will feel perfectly well with blood glucose concentrations in the mid or upper teens (mmol/l) for long periods. Random blood glucose measurements can be very misleading, especially in insulin treated patients, since considerable fluctuations often occur even during a single day. Quite different conclusions might be drawn from blood taken at 9.30 am soon after breakfast (when blood glucose might be over 20 mmol/l) and at the end of an afternoon clinic (when blood glucose in the same patient might be lower than 3 mmol/l). The other conventional guide to diabetic control, home urine testing, is also unreliable. Although there are highly motivated patients who bring to the clinic carefully recorded results of regular testing, done at specific times, others are in the habit of simply testing "when I feel like it" or not at all. Even reliably collected results may still be misleading because the renal threshold is not constant, so that the meaning of trace, 1%, and 2% varies from patient to patient,[82] with urine volume, and with many other variables. Also, once below the threshold, hypoglycaemia cannot be differentiated from normoglycaemia.

In young and middle aged patients, who are at risk of long term complications, the aim should be to restore blood glucose concentrations close to the normal range.[1] Blood glucose should certainly be kept below 10 mmol/l most of the time (even after meals), with preprandial concentrations between 4 and 7 mmol/l. In other words, glycosuria should be abolished altogether. Such a target will not be appropriate for older patients, and indeed may not be attainable in some younger patients.

Of course, simply measuring Hb A_1 cannot by itself realise these standards of good control, but the result can indicate whether or not success has been achieved. Really close control, such as described in the last paragraph, will usually require home blood glucose monitoring to guide adjustment of food intake and insulin doses.[3] Hb A_1 indicates only whether overall control is good or bad; when it is bad Hb A_1 results do not indicate precisely what is wrong—that is, at what time(s) of day blood glucose is high. Nor, as mentioned earlier, will they specifically indicate that hypoglycaemia has occurred, though there may be clues from an apparent disparity between low Hb A_1 and other evidence that control is poor. Therefore Hb A_1 and home

blood glucose monitoring should be looked at together, each complementing the information provided by the other.

Because of variation between the shape of diurnal glucose profiles in different patients, Hb A$_1$ results cannot be directly translated into average blood glucose concentrations. However, the patient whose Hb A$_1$ is below 10% of total haemoglobin (in our laboratory the normal range in non-diabetic subjects is roughly 5–9%) deserves a pat on the back and encouragement to keep up the good work; those with results between 10% and 14% "could try harder," and those over 15% are in need of remedial therapy—assuming of course that good control is being aimed for (in older patients relief from symptoms rather than the attainment of normoglycaemia should be the goal of treatment). When Hb A$_{1c}$ is being measured by a specific method, the ranges of good, bad, and indifferent will be lower.

Svendsen *et al*[83] studied the relation between mean blood glucose and glycosylation in patients with particularly stable control, and concluded that Hb A$_{1c}$ = 2·07 × (mean blood glucose)$^{0·596}$. Such mathematical precision does not apply to most patients, whose control is more variable.

Several authors[84–86] have formally compared the usefulness of glycosylated haemoglobin with other indices of diabetic control when both were measured regularly. Borsey *et al*[86] concluded that a single measurement of Hb A$_1$ in non-insulin-dependent patients was as accurate as the mean of three successive monthly glucose estimations, because the former varies relatively little from the average value. Variation of a random blood glucose measurement from the mean is of course likely to be much more extreme in insulin treated patients, and hence Hb A$_1$ measurements are all the more useful.

PREGNANCY
In view of the undoubted impact of diabetic control on fetal well being, it is especially important to aim for and achieve normoglycaemia during gestation (and indeed before conception). Home blood glucose monitoring has allowed women to stay at home up to the time of delivery,[87] overcoming the need for admission to hospital for several months to check blood sugar, which used to be routine practice. In these circumstances it is reassuring to all concerned to see regular Hb A$_1$ measurements within the normal range, and they are very useful.

Schwartz *et al*[88] reported that Hb A$_{1c}$ values in diabetic women were reduced during pregnancy. It was not clear whether this was due to metabolic consequences of pregnancy, better medical management during pregnancy, or other undefined factors—for example, an increased proportion of young red cells in the circulation due to accelerated erythropoiesis. Kjaergaard and Ditzel[89] also found lower Hb A$_{1c}$ concentrations during pregnancy, but their close relation to lower blood glucose suggested that better treatment rather than any other factor was responsible. Certainly Hb A$_1$ concentrations during pregnancy show a positive correlation with birth weight (corrected for gestational age),[90] which is consistent with established experience that large babies are associated with poorly controlled diabetes.

There are changes in glucose tolerance during pregnancy even in non-diabetic women, so that the "normal range" for Hb A$_1$ in pregnancy needs to be established separately.[91]

DIAGNOSIS[92–94]
The disadvantages and sources of error in performance of the glucose tolerance test are well established,[95] but considerable confidence in the precision and reliability of a single Hb A$_1$ measurement is required before this test becomes the arbiter of diagnosis. Furthermore, since there are many different methods in use and no agreed reference range, a national or international standard is not a viable proposition for the time being.[61]

Conclusions

By providing an index of diabetic control that effectively integrates blood glucose concentrations over several weeks, measurement of glycosylated haemoglobin offers a new insight into metabolic abnormality that is characterised by wide fluctuations. Currently, the potential of glycosylated haemoglobin is limited in practice by methodological problems, the various assay methods available all having their drawbacks. The most convenient methods are subject to errors that may introduce considerable imprecision, and comparison between results obtained by different methods is not always straightforward. It is likely that these difficulties will be overcome by greater experience, by technical improvement and modification of assay methods, and by the wider availability of stable standards. The importance of glycosylation is firmly established and measurement of Hb A$_1$ is here to stay.

Robert Tattersall, whose great help and influence have been crucial to all our work.

This paper was prepared at the invitation of the Association of Clinical Biochemists' working party for clinical laboratory investigation. I gratefully acknowledge the help of Dr DJ Worthington and Mr EF Legg in the preparation of the manuscript.

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