



Testing assumptions about glycohemoglobin with a retrospective time-resolved test

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ABSTRACT

A method of dividing red blood cells (RBCs) of whole blood into age-related fractions enables the time-resolved analysis of glycohemoglobin (GHb) accumulation. The time series is validated by pyruvate kinase, an enzyme whose activity is known to decrease steadily as RBCs age. The time series plot of GHb, expected to be linear, instead resembles an exponential decay curve that may be normalized with a mathematical transform. Significant discontinuities in such curves, even after transform, occurred in 47 of 101 patients with elevated plasma glucose and corresponded with clinical histories of changes in blood sugar control. Time-resolved tests of GHb demonstrate deviations from the linear plot in 31% (167 of 538) of patients. Almost one-third of patients fail to meet assumptions made while interpreting GHb, i.e., a steady state of either blood sugar or RBC turnover. Also, the assumed irreversible formation of GHb may not be supportable. GHb results must be interpreted in light of the physiological status of the patient. Although GHb is used as a model, other analytes may be amenable to retrospective time-resolved analysis from the same density fractions of RBCs. *Lab Hematol* 4:142-148, 1998

KEY WORDS: Time resolution · Steady state · Amadori rearrangement

INTRODUCTION

A widespread assumption is that hemoglobin is continually glycosylated very gradually over the 120-day lifespan of a red blood cell (RBC) [1,2]. The glycohemoglobin (GHb) accumulation is believed closely related to the patient's blood sugar history [2-6]. Because the accumulation is believed irreversible, the blood sugar history is preserved over the circulating lifespan of RBCs. The potential loss of

measurable GHb—through reversal of the Amadori rearrangement—has not been widely considered and may, for example, explain deviations from expected linear accumulation of GHb.

A clear understanding of the physiological and biochemical assumptions underlying GHb testing aids the interpretation of clinical results. As currently obtained, the single point GHb test result can be difficult to interpret because of certain general assumptions. Listed in order of the greatest impact on interpretation, these assumptions follow:

- the patient is in steady state with respect to blood sugar;
- the patient is in hematological steady state;
- the GHb, once formed, never reverts to a nonmeasurable form;
- significant glycation of hemoglobin is limited to the N-terminal valine of the hemoglobin β chain, producing HbA_{1c}; and
- all laboratory methods give the same results.

With the advent of the retrospective time-resolved test [7], it is possible to evaluate the first three assumptions. In this study, the reversible nature of the Amadori rearrangement is evaluated, and the first two assumptions are reviewed in light of clinical results. The fourth assumption is in error because lysine residues along both α and β chains may be glycosylated [2]. Some methods detect all glycation, others detect only HbA_{1c}, i.e., specifically the glucose glycosylated terminal valine. Manufacturers could resolve the issue by providing reference standards with both GHb and HbA_{1c} values. Conversion values of their results to a standardized procedure would be useful [1,6]. The fifth assumption will not be addressed here.

MATERIALS AND METHODS

Samples

Five hundred thirty-eight blood samples from both diabetic and nondiabetic patients were obtained for the study. The majority of samples were obtained in a hospital laboratory setting after hematology testing, with only the blood glucose value used as a guide for sample selection. These were remnant samples taken after other testing. For a subset of patients, histories were obtained to compare with study findings.

Additional samples from a group of 76 patients with non-insulin-dependent diabetes mellitus (NIDDM) were evaluated under a separate protocol at monthly intervals for 5 months. Daily

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blood glucose readings and other clinical data were available for these patients. Case histories of certain patients will be discussed below, but complete details of this study will be separately reported. Informed consent was provided by these patients.

Sample Preparation and Chemical Analysis

RBCs were separated into 10 density fractions as described previously [7]. The process is analogous to cutting 10 ordered fractions from the RBC stack of a microhematocrit after centrifuging at 15000g. The fractions correspond to the length of time that the RBCs have been in circulation [7]. This is supported by the gradual reduction of pyruvate kinase (PK) activity from one density fraction to the next, in descending order [8,9]. Exceptions to this age ordering of RBCs occur when there is a rapid replacement of RBCs. In rare instances, a cohort of cells with a different mean cell hemoglobin concentration (MCHC) may exist in a patient (see example in Fig. 1).

Routine measurements recorded for each sample included:

- GHb% on whole blood (all samples)
- GHb% on each of 10 fractions (all samples)
- total Hb on each of 10 fractions (all samples)
- PK on whole blood (300 samples)
- PK on each fraction (300 samples)

All measurements were performed as previously described [7]. Briefly, GHb was measured by minicolumn affinity chromatography using GlycoGel II (Pierce, Rockford, IL). This gel is an agarose matrix containing side chains of aminophenyl boronic acid on a 6 carbon spacer. The 76 samples that were evaluated on 5 monthly occasions were measured by using the same affinity matrix on a semi-automatic instrument (Helena Laboratories, Beaumont, TX). Total Hb was estimated by its absorbance as measured by its optical density (HbOD) at 415 nm. PK was evaluated as a reaction coupled to lactate dehydrogenase with a reduced form of α -nicotinamide adenine dinucleotide (NADH) utilization as the end point, according to the method of Seaman et al. [8]. Between-run stability was limited by reagent stability. Within-run reproducibility, after averaging duplicate determinations, was acceptable for our purposes.

A slide for hematological examination was prepared for a large subset of samples. Occasionally, it was helpful to examine this slide to troubleshoot a question or problem that arose during evaluation of the data.

Mathematical Model and Transform

According to Seaman et al. [8], the time line may be reconstructed from a series of density fractions of RBCs by accounting for the total Hb in each fraction. By adding the absorbances of each fraction (HbOD), the total 120 days is represented. The HbOD of each fraction divided by the total HbOD equals the number of days represented by that fraction. The time ordering of fractions so obtained and how it is used in the present work is shown in Table 1 and its companion, Figure 2.

Table 1 and Figure 2 also show the method used to evaluate the assumption of nonreversible glycation of hemoglobin. The model, which is similar to that of Bunn et al. [2], is presented here.

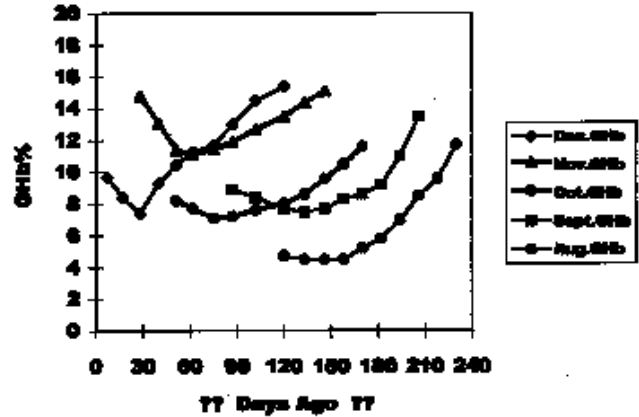
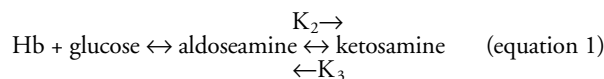


FIGURE 1. This unpredicted time-resolved GHb series is due to a set of hypochromic RBCs that are less dense than the patient's remaining cells. These hypochromic RBCs always migrate to the top density fractions and may be mistaken for the most recently produced ones, which should have the least GHb accumulated. As these cells age, however, they accumulate GHb and form this unusual pattern. In the December sample, steady state has been nearly restored.

$$\text{GHbT} = \text{GHb} (1/e^{-kt}) \quad (\text{equation 2})$$

where:

- Hb is the initial hemoglobin
- aldosamine is a Schiff base, which is known to be reversible
- GHbT is the transformed glycohemoglobin
- GHb is the measured glycohemoglobin (a ketosamine)
- e is the base of the natural logarithm
- k is an experimentally derived constant that is equivalent to K_3
- t is the time (in days) computed from total Hb for each fraction

The constant k represents the rate of reversibility of the Amadori reaction. Note that if $k = 0$, then $\text{GHbT} = \text{GHb}$. In this study, the equation was implemented in a spreadsheet using the data from six patients. The value of k was experimentally varied until a plot of GHbT against t produced a straight line. Although the level of GHb and blood glucose varied among these patients, all six were known to be in a steady state. Three patients were neither anemic nor diabetic, two patients had moderately elevated HbA_{1c} and blood glucose, and one patient consistently had very high blood glucose and a GHb in the very high abnormal range. These patients are represented in Figure 3 by A, B, and B+, respectively.

RESULTS

General Interpretation of Data

Density fractionation of whole blood without artificial gradients provides an opportunity to measure experimentally the Hb and GHb subsets of RBCs, based on their age in circulation. Table 1 shows an example of how data are treated to develop the time line and how to calculate the value of GHbT. For each of ten density fractions, Hb, PK, and GHb are measured directly [7]. The measured HbOD for

TABLE 1. Example (sample no. 542) of analysis of red cell fractions by Hb and GHb using the linear transform of GHb^a

Fraction	Data			Calculations			SGHb ^h
	HbOD ^b	GHb ^c	PK ^d	Days ago ^e	1/e ^{-kt} ^f	GHbT ^g	
1	0.283	13.62	31.7	9.68	1.04	14.16	14.16
2	0.320	17.28	22.9	20.62	1.10	19.00	16.58
3	0.339	18.70	21.5	32.21	1.16	21.69	20.35
4	0.369	19.95	18.8	44.83	1.22	24.34	23.01
5	0.383	19.39	15.8	57.93	1.30	25.21	24.77
6	0.313	19.17	16.6	68.63	1.36	26.07	25.63
7	0.314	19.26	14.8	79.37	1.43	27.54	26.80
8	0.374	19.37	13.2	92.16	1.51	29.25	28.39
9	0.423	19.13	10.9	106.63	1.62	30.99	30.12
10	0.391	19.93	11.2	120.00	1.72	34.28	32.63
Total	3.509						
Mean		18.58	17.741				
Whole blood		17.70	21.20				

^aThe linear transform is based on equation 1.

^bEach of the ten fractions has a volume of ~10 L. Each is diluted with a lyse solution to 2 mL, and optical density (HbOD) is measured at 415 nM.

^cGlycohemoglobin (GHb) is measured by minicolumn affinity chromatography, using Glycogel II (Pierce, Rockford, IL).

^dPyruvate kinase (PK) is measured by the method of Seaman et al. [8]. Activity is in relative units held constant for each sample.

^eDays ago is a calculation of the circulating age of RBCs in the fraction, as described in the text.

^f1/e^{-kt} is the calculated transform factor where t = days ago and k = -0.0045.

^gGHbT is the calculated GHb according to equation 1 and is the product of GHb and the transform factor.

^hSGHb is a first degree smoothing of GHbT, which is accomplished by averaging each two adjacent values of GHbT.

all fractions is added together and the total divided by 120 to establish a factor for each fraction, which is normalized to the full life span of a RBC. The measured HbOD for each fraction is divided by the normalizing factor to establish the number of days represented by the fraction. For the first (youngest) fraction, this calculation establishes

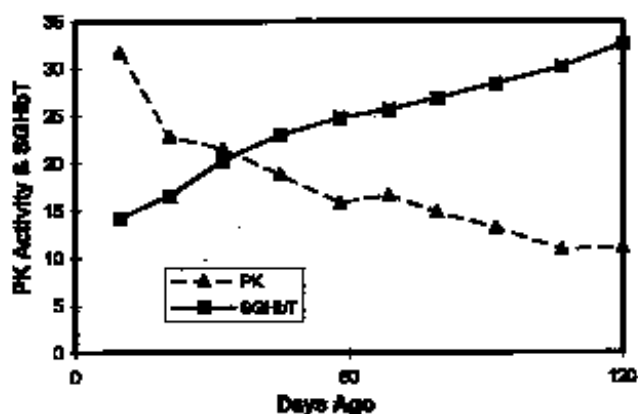


FIGURE 2. A graphical translation of the data and calculations of Table 1. The GHb data presented in the other figures were developed in the same way. In this 42-year-old patient's clinical history there was no indication of diabetes until the admission for surgery for an infected pilonidal cyst. The plasma glucose value at the time of admission was 1600 gm/dL. PK values are given in an arbitrary activity scale.

the days that the RBCs have been in circulation before taking the sample. For subsequent fractions, the circulating age, in days, is calculated by cumulating the days from fraction 1. Thus, the final fraction of a sample is defined as being in circulation for 120 days. (Note that these designations are arbitrary because one may just as easily take the midpoint or average of each fraction to get a slightly more precise date.) Figure 2 is a graphic presentation of Table 1. All data were analyzed in this format. This scheme of establishing the circulating age of RBCs is based on the work of Seaman et al. [8], who also required the mean cell volume (MCV) of density-separated fractions to follow an orderly pattern. Confirmation of this orderly pattern of MCV for the present work has been reported, using an automated hematology analyzer (H.1, Technicon, Tarrytown, NY) [10].

The constant k from equation 2 was varied experimentally from 0 to -1. Negative values were used because the constant represents the reverse reaction of the Amadori rearrangement [12]. For the present study, k was held constant from fraction to fraction and from patient to patient to test the hypothesis that a single feature (i.e., the reversibility of the Amadori rearrangement) accounts for the majority of apparent nonlinearity. Note that k could be varied from one fraction to another, but that the result would indicate more than one chemical factor or physiological factor at work. When k = -0.0045 (see Table 1), the curve became a straight line for samples known to meet the steady-state assumptions, i.e., when daily blood sugar measurements are constant and the hematological parameters or microscope slide evaluations are within normal limits. This is further discussed below.

Because there was a known imprecision in the measurement of GHb, adjacent values were averaged to provide a first degree of smoothing (SGHb) in the final column of Table 1. (As the method

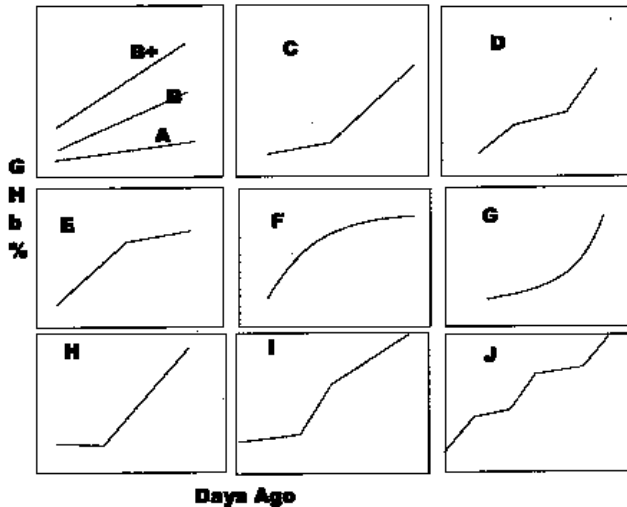


FIGURE 3. Patterns of glycohemoglobin accumulation (corrected for slight reversibility of the Amadori rearrangement) that were considered theoretically possible. For each diagram, the horizontal axis is time in days, extending from the day the sample was taken and backward 120 days at full scale (Days Ago). The vertical axis is the transformed glycohemoglobin (GHbT) at each time period. The interpretations are as follows: A, normoglycemic individual; B and B+, hyperglycemic, but in steady state for glucose; C, hyperglycemic, except much improved most recently; D, usually hyperglycemic, but with one normal episode or hypoglycemia 2 months ago; E, initially in good control, but with a sudden change to hyperglycemia; F, initially in good control, then gradually changing to hyperglycemia (expected in newly developing diabetes); G, initially hyperglycemic, then gradually improving toward normal (expected with good weight control in some patients with NIDDM); H, completely flat segments are seen only in severe hypoglycemia or after a sudden increase in red cell production; I, usually in good control, but with one episode of hyperglycemia; J, continually changing episodes of good control, hyper-, and hypoglycemia. Note that the pattern in H is made according to data in the fractions. In this case, however, the time scale is incorrect because the assumption of steady-state hematology is not valid. Time scale is also difficult to interpret when increased RBC production is in steady state (e.g., compensated hemolytic anemia or pregnancy, theoretically when the pattern would trace a linear plot with a lower slope (A-)).

within the laboratory improved and especially after implementing the semi-automatic method, the smoothing was no longer required, but was continued to maintain consistency of data.) These smoothed values (SGHb) are plotted on the vertical axis of Figure 2, while the circulating age of RBC is plotted on the horizontal axis as Days Ago.

Overview Data Summary

Theoretically, clinical histories in diabetes and RBC turnover should translate into a limited set of time-resolved graphical pat-

TABLE 2. Summary of time-resolved GHb patterns, compared with a currently standard GHb test

GHb class ^a	Whole blood time-resolved test					
	Standard shape of plotted GHb results			Total		
	Linear ^b	Discontinuous ^c	Curved ^d			
		1+ ^e	2+ ^e	3+ ^e		
Normal	18	9	13	3	2	45
Intermediate	6	6	12	5	—	29
Abnormal	8	3	9	4	3	27
Totals	32	18	34	12	5	101

^aGHb class, how a diagnostician would classify the standard GHb of current practice on the same patient.

^bLinear, equivalent to patterns A, B, and B+ of Figure 3.

^cDiscontinuous, equivalent to patterns C, D, E, H, I, or J of Figure 3.

^dCurved, equivalent to patterns F or G of Figure 3.

^ePlus sign (+) indicates visual severity of discontinuity in the accumulation curve. 1+, close to linear. 2+ and 3+, considered significant.

terns. The 11 most likely patterns were set down before a large set of data was collected. These are depicted in Figure 3, with a general interpretation of each in the legend. With very few exceptions, which are noted below, the observed patterns fit the predictions of Figure 3. An A- pattern, which would have a slope lower than that in A, has been found in 8 samples taken from pregnant patients who were at 10 to 16 weeks gestation. Table 2 summarizes the patterns observed in the first 101 random samples in which the blood glucose was elevated to at least twice the normal level. Clinical summaries were available for these patients and, in each case, the discontinuities corresponded to clinical episodes of short-term hypo- or hyperglycemia, or of gradual changes as discussed below. Table 3 is a summary of all 538 samples that are classified by the patterns shown in Figure 3. If blood loss during the menstrual cycle was sufficiently heavy, a J-type curve would be expected. The data contained too few women of childbearing age to confirm this expectation.

Time Line

Justification of the time line is provided by demonstrating linearity of the PK plot against Days Ago in 293 of 300 patient samples. In seven exceptional cases, described below, clinical histories confirmed that the patient was not in a hematological steady state. However, many of the patients with linear PK plots, who were considered to be in hematological steady state, did not have straight line plots of either GHb or SGHb against the same time line. Hence, steady state for blood sugar, using fractionated cells and GHb, may be evaluated separately from the steady state for hematology, using the same time line.

Further Observations on PK

Of the seven patients whose PK plots were nonlinear with the time line, two had sudden and effective correction of nutritional anemia. Three were postsurgical with severe blood loss several days before the blood sample was taken. Another patient had both nutritional correction and blood loss. A final patient had a complex clinical history, including glucose intolerance related to chronic pancreatitis and severe hemorrhage surrounding the pancreas. All

TABLE 3. Summary of glycohemoglobin patterns classified according to Figure 3

Curve type	A-	A	B	B+	C	D	E	F	G	H	I	J
No. observed	12	55	172	133	30	38	36	10	8	8	10	26

seven patients exhibited the pattern in Figure 3H. In a separate patient sample, the PK results were linear but very much elevated and almost horizontal. The patient who provided the sample suffered from an autoimmune hemolytic anemia. The corresponding GHb plot was linear.

Justification of the Transform

In 73 of 76 patients from whom monthly samples were taken (for 5 months), daily glucose tests indicated a steady state of blood sugar. A plot of measured GHb in these patients against the time line invariably presented a curve resembling the pattern in Figure 3F. Application of the transform to these data and replotting resulted in conversion to straight lines as depicted by patterns A, B, and B+ in Figure 3. The slope of the straight line correlated with the recorded daily blood glucose test.

Additional Observations of Time-Resolved GHb

Two patients in the group of 76 had significant deviations from steady-state levels of both daily blood sugar and time-resolved GHb (TRG). These deviations spread through the time-resolved plots of later obtained samples, e.g., a segment with a lower slope appeared 30 more Days Ago a month later as in the progression in the horizontal from Figure 3C to 3D.

In one patient, whose progress was followed for 5 months, the GHb accumulation pattern was highly unusual and did not fit any of the eleven predicted patterns (Fig. 3). However, the curves were internally consistent with each other and demonstrate that time-resolved curves are amenable to physiological interpretation. The patient's history includes an episode of uremia in the month before the first sample was taken. A set of hypochromic RBCs were produced during this episode. These cells, which have a lower density than the patient's remaining RBCs, always came to the top of the red cell stack during equilibrium centrifugation, thus distorting the age fractionation pattern. However, this cohort of cells continued to accumulate GHb.

In such exceptions, a time course cannot be plotted because RBCs do not separate into age cohorts by density layers. Once the hypochromic cells left circulation, a more normal pattern resumed (December of Fig. 1). In this exceptional case, one notes higher GHb in earlier fractions while the hypochromic cohort of RBCs matured. Unfortunately, PK testing was not done on this patient's samples. Review of hematological slides, however, confirmed presence of these hypochromic cells for the first 4 months and their absence in the last month. For the entire 5 months, the patient was in glycemic steady state according to daily home glucose testing records.

Using $k = -0.0045$ with the transform also produced a linear plot for 355 of 521 patients in the larger study. There was a better fit for a linear plot using $k = -0.009$ in the eight women who were between 10 and 16 weeks pregnant. The difference may be due to the known hemodilution that accompanies pregnancy. However, more samples are needed to confirm this difference from the rest of the study.

Interpretation of Time-Resolved GHb Plots

Two significant features of the plots in Figure 3 are the slope of the line and the presence or absence of discontinuities. The slope approximately correlates with average blood glucose during the period represented. Straight lines (no discontinuities or curvature) correspond to a steady state at any given level of blood sugar.

Discontinuities and curvatures of GHb accumulation in TRG curves are usually associated with episodes of changing blood sugar levels. Often these episodes are difficult to extract from patient histories. Some illustrative case histories have been published [7]. Many others have been accumulated. For example, six of the ten patients with the pattern of Figure 3F were from newly diagnosed diabetes mellitus patients. However, one insulin-dependent diabetic patient with this pattern experienced gradually increasing emotional distress in the 4 months before her wedding and did not change her diet, exercise, or medication. The Figure 3C pattern is often observed 1 or 2 months after a specialist or nurse practitioner takes responsibility for the care of a diabetic patient in poor control.

DISCUSSION

Patients in a steady hematological state with well-controlled blood glucose levels appear to glycate Hb in a linear fashion over time. Data strongly suggest that GHb, once formed, may revert to Hb or become nonmeasurable for other reasons. The relationship of circulating RBCs to measured GHb, without application of the transform, invariably looks like the curve in Figure 3E. A very experienced interpreter could develop a mental set of curves analogous to those in Figure 3, but without application of a transform. However, most of us are linear thinkers and would find this a difficult exercise. When examining transformed data plotted for each patient, special attention is given to remaining curvature and discontinuities in the accumulation curves. However, the transform, which accounts for the loss of measurable GHb, contributes to more than just easy interpretation of results [11].

In the present study, patients known to be in steady state show a linear relationship between time (t) and transformed GHb (GT). About 30% of all patients included in this study could not be assumed to have steady-state blood sugar when represented by a TRG. These patients may have compensating episodes of hyper- and hypoglycemia so that a single-point GHb may appear normal. Indeed, 18 of 45 patients with single-point GHb in the normal range, actually had such episodes of hypo- or hyperglycemia verified by clinical history (Table 2). Pattern H in Figure 3, seen in the early stages after accelerated blood replacement, represents a kind of blood dilution leading to lower than expected levels of GHb. A standard single-point GHb would give misleading low results. Similar anomalies are expected with the blood dilution that occurs with pregnancy. Without careful further study, differences in patients as they are gradually improving, getting worse, or suffering severe blood loss may be missed and the opportunity to investigate periods outside the steady state lost.

An explanation for this loss of measurable Ghb is suggested in the literature. Bunn et al. [12] did a prospective study with two volunteers who were pulse-labeled with radioactive Fe^{55} ferritin. The specific activity of GHb continued to rise; the specific activity of total Hb was essentially constant for over 100 days, until the RBC cohort began to be resorbed. However, the rise of GHb-specific

activity became less steep as the cohort of radioactive-labeled RBCs approached the age at which they are resorbed. Bunn et al. [12] produced three mathematical models based on equation 1.

The first model assumed $K_3 = 0$, and showed that the rise of specific activity in GHb should be continuous and linear. A second model assumed $K_3 > K_2$. In this case, the specific activity should rise rapidly to some equilibrium and then remain constant until the cells are resorbed. This is the model also supported by Mortensen [13]. The third model assumed $K_2 > K_3 > 0$. In this case, the continual rise in the specific activity curve of GHb should have a downward concave shape. Experimental data fit the third model with $K_3 = 0.01$. The reversibility of this Amadori rearrangement was considered small and has been ignored [1–4]. Even Higgins et al. and Bunn et al., in later publications, decided to ignore the reversibility of the Amadori rearrangement [14,15].

The results of the present study suggest that the continuous accumulation of GHb is mitigated in part by loss because of the slowly reversible reaction. The loss is amenable to calculation, and, as a result, a more precise history of blood sugar control may be presented. The loss calculated from presented data, $k = -0.0045$, is very similar to that calculated by Bunn et al. in his prospective study of two volunteers [12]. Although the loss is considered negligible by others, this study shows that approximately 70% of formed GHb in the oldest fractions is no longer measurable (Table 1) because of the exponential decay for more than 100 days [15].

Rigal et al. [16] observed that GHb is unusually low in the presence of hemolytic anemia and in the absence of diabetes. This phenomenon is but one of many anomalies that may be demonstrated when GHb is time-resolved. It seems important to evaluate the physiological lessons learned in the present study. However, it is not my purpose to convince readers to change practice or to request time-resolved glycohemoglobin tests for all patients. In fact, the test is not yet on the market. Rather, I suggest that consideration of physiological factors enhances the interpretation of single-point GHb results. Three examples follow.

First, transformed GHb of all fractions may be averaged and this average compared to that of non-transformed measurements. With these steps, correlation is very good and has a slope of approximately 1.3. This means that if the process of measurable glycation were truly irreversible, the true value would be 30% higher in a patient in steady state.

Second, correlation of average GHb with blood glucose was examined in a subset of 53 patients: 25 had straight line TRG patterns and 28 had significant discontinuities. The range both of blood glucose (100–640 mg %) and of GHb (5–25%) was similar in the two subgroups. The correlation coefficients were $r = 0.35$, 0.69, and 0.14 for the whole group, the group with straight TRG plots, and the group with discontinuous TRG plots, respectively. These results may explain why devising general regression equations connecting blood sugar to GHb has been a difficult and often imprecise activity [2–6]. However, with density separation one is likely to find one region where correlation improves [9].

Third, one of the most interesting findings with a retrospective time-resolved test for GHb is the occurrence of a curve patterned after that seen in Figure 3F. In 6 of 10 patients in this study, there was no prior suspicion of sugar intolerance. The other four were known diabetic patients who were changing gradually. Without adjustment for a reversible Amadori rearrangement, all 363 straight GHb accumulations would have a downward concave appearance.

It is only by making the transform that the Figure 3F curve, with its downward concavity, stands out as an indicator of gradual change.

Based on this study of GHb as a retrospective time-resolved test, each clinical GHb result must be evaluated with the following three questions in mind. Has there been any indication of either blood loss or accelerated blood replacement in the past 4 months? Is there reason to believe that a dramatic change in blood sugar control is in progress? A positive answer to either of these two questions will make interpreting GHb so difficult that the test may as well not be done. Pregnancy falls into this category. Perhaps recent intensive therapy also fits this category. Has there been a slow change in blood glucose over 4 months? If so, a decrease in blood sugar is usually more dramatically represented by serial GHb measurements because it acts in concert with the reversible Amadori rearrangement. The opposite may be true for an increase in blood glucose; the higher equilibrium is reached slowly. With either type of change, interpretation is difficult because humans tend to be linear thinkers. Indeed, a sophisticated but uncooperative patient could easily determine that a pig out 4 months before the next GHb test would have only a 30% effect on the result compared with the same indiscretion just before the test is performed, as currently practiced.

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