with chronic stable asthma responds appropriately to hypoxia. We suggest that in the presence of chronic hypoxia the value of PaCO_2 taken as "normal" should be that predicted by the relation of PaCO_2 to PaO_2 shown in figure 1—that is, a PaCO_2 of 5.3 kPa (40 mm Hg) would be normal if PaO_2 were 13.3 kPa (100 mm Hg) but abnormally high and indicating hypoventilation if PaO_2 were low. The "normal" PaCO_2 would be 3.7 kPa (28 mm Hg) at a PaO_2 of 6.7 kPa (50 mm Hg).

We considered that patients who are severely ill from asthma might not show the acclimatisation response to hypoxia because respiratory control is abnormal or because of severe mechanical impairment to ventilation. Rees *et al*³ reported arterial blood-gas values for patients with status asthmaticus. We examined the relation of PaCO_2 to PaO_2 by plotting the values obtained in their patients on the day of admission (fig 2). In all patients PaCO_2 was above the value predicted from our patients with chronic stable asthma. Many of the patients of Rees *et al* would already be regarded as severely ill on the basis of their arterial blood-gas and pH values. There remains a group with a PaCO_2 of about 5.3 kPa (40 mm Hg), however, in whom we suggest ventilatory control was impaired. These patients had therefore failed to adjust their ventilation normally, even though most of them had probably been severely ill and hypoxic for several days.⁶⁻⁸

In conclusion, we suggest that the relation between PaCO_2 and PaO_2 in patients with chronic stable asthma (PaCO_2 predicted = $0.23 \text{ PaO}_2 + 2.2 \text{ kPa}$ (or 16.6 mm Hg)) be adopted as a criterion for assessing adequacy of ventilatory control. Values of PaCO_2 above this predicted normal indicate impaired ventilatory control and may give early warning of impending decompensa-

tion in patients severely ill from asthma even when the arterial pH is normal.

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Changes in glycosylated haemoglobin after poor control in insulin-dependent diabetics

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Summary and conclusions

Glycosylated haemoglobin (HbA_{1c}) was measured in seven insulin-dependent diabetic patients before, during, and after a seven-day period of monitored poor control. There was considerable individual variation in the pattern and degree of change in HbA_{1c} concentration induced by poor control and the time when it occurred. Greater increases in HbA_{1c} were seen during the period of metabolic derangement than in the subsequent two months.

More information is required before HbA_{1c} estimations are widely used clinically to monitor control in individual diabetics.

Introduction

Concentrations of glycosylated haemoglobin (HbA_{1c}) are raised in diabetic patients and, since the glucose linkage is considered

to be relatively stable, are thought to reflect the mean blood glucose concentration during the preceding one to two months. Hence HbA_{1c} estimations are being used as a means of monitoring the degree of overall control of blood glucose achieved in individual diabetics. There are, however, many problems associated with measuring HbA_{1c} and neither the time relation between changes in blood glucose and HbA_{1c} concentrations nor the stability of HbA_{1c} nor its sensitivity in detecting poor control has been clearly defined.

We compared two common methods of estimating HbA_{1c} and examined the change in concentration of HbA_{1c} resulting from a period of poor control deliberately induced in longstanding, insulin-treated diabetic patients maintained under strictly monitored, metabolic conditions.

Patients and methods

One male and six female diabetic patients being treated with insulin were studied (table I). All were fully ambulant, none was obese, and none had retinopathy, neuropathy, or nephropathy. All gave informed consent to the investigation.

The study covered 13 weeks and was divided into three periods. In the initial three-week assessment period patients were at home following their usual diet and insulin regimen and undertaking normal activity. They were visited each week on the morning after completing a 24-hour urine collection for measuring urinary glucose excretion, and fasting blood was taken for estimating whole blood true glucose, serum lipid, and HbA_{1c} concentrations.

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TABLE I—Clinical details of patients studied

Case No	Age and sex	Duration of diabetes	Duration of Insulin therapy	% Of standard weight	Diet (MJ)	Insulin dose (units)		
						Normal	Poor control	% Reduction
1	72 F	6	3 yr	86	8.36	20 Soluble, 28 isophane	14 Soluble, 14 isophane	50
2	76 F	3	6 mth	85	7.52	16 Mixtard	5 Mixtard	69
3	67 M	12	9 yr	102	9.20	16 Soluble, 22 PZI, 12 Soluble	8 Soluble, 12 PZI, 6 Soluble	48
4	74 F	1	8 mth	80	7.52	24 Soluble, 20 isophane	7 Soluble, 6 isophane	70
5	76 F	4	4 yr	70	5.85	28 Lente	14 Lente	50
6	57 F	18	6 yr	91	5.02	20 Soluble, 20 isophane	4 Soluble, 4 isophane	82
7	60 F	3	3 yr	89	6.69	6 Soluble, 16 isophane, 22 isophane	3 Soluble, 11 isophane, 8 isophane	50

Conversion: SI to traditional units—Diet: 1 MJ $\approx$ 239 kcal.

TABLE II—Patterns of change in HbA₁ concentrations related to change in diabetic control

Case No	Changes in HbA ₁ (highest single value during these periods expressed as a percentage of the mean for weeks 1-3)			Mean 24-hour Urinary glucose (mmol)		Mean change in fasting blood glucose (mmol/l)*	Lowest plasma bicarbonate (mmol/l)
	Weeks 4-5	Weeks 8-9	Weeks 11-12	Weeks 1-3	Weeks 4-5 (days 3-10)		
1	169	117	88	3.4	141.9	4.0	26.0
2	174	116	112	49.4	204.4	1.2	26.0
3	113	93	88	16.5	441.7	6.8	19.0
4	108	78	81	4.7	10.5	-4.4	23.5
5	120	112	100	32.5	376.2	15.8	25.0
6	115	92	135	3.3	85.9	3.7	24.5
7	136	92	—	85.3	392.6	6.3	25.5

*Difference between mean fasting blood glucose concentration for weeks 1-3 and days 3-10 of weeks 4 and 5.

Conversion: SI to traditional units—Blood glucose: 1 mmol/l $\approx$ 18 mg/100 ml. 24-hour urinary glucose: 1 mmol/24 h $\approx$ 0.18 g/24 h. Bicarbonate: 1 mmol/l $\approx$ 1 mEq/l.

During the second phase of the study patients were admitted to a metabolic unit for 14 days. They continued to take their normal diet, daily 24-hour urine collections were made for measuring urinary glucose, and daily samples of blood were taken fasting and two hours after the main midday meal for estimating glucose, HbA₁, electrolyte, and urea concentrations. Fasting serum lipids were measured on the first, seventh, and 14th day of this period. For the first seven days after admission patients received about half their normal daily dose of insulin (mean reduction 59%; range 48-82%). In the next seven days diabetic control was restored and the patients were then discharged home taking their usual dose of insulin.

In the next eight weeks patients followed their usual diet and insulin and undertook normal activity. They were again visited at home each week on the same morning of the week as in the initial assessment period. As before, each made a 24-hour collection of urine for measuring glucose, and fasting blood was taken for estimating glucose, lipid, and HbA₁ concentrations.

METHODS

Whole blood and urinary glucose concentrations were measured by an automated glucose oxidase method. Serum cholesterol, triglyceride, and phospholipid concentrations were measured using commercial kits (Boehringer Mannheim), and lipoprotein electrophoresis was performed on cellophane.

Total glycosylated haemoglobin—that is, HbA_{1a+b+c}—was estimated in each blood sample by both a colorimetric and a chromatographic method. Blood samples for measuring HbA₁ were handled in exactly the same way for inpatients and outpatients. They were collected into tubes containing EDTA and remained at room temperature for up to one hour after collection. The amount of HbA₁ measured by either method did not change significantly when blood samples were left standing at room temperature for up to four hours instead of being refrigerated immediately. Thereafter, the storage procedure depended on the method of analysis. Samples for chromatographic analysis were refrigerated (for up to 24 hours) until the red cells were washed and a haemolysate prepared. The haemolysate was

then frozen (for no longer than seven days) until analysis. Samples for chromatographic analysis were simply refrigerated (for no longer than six days) before analysis. The amount of HbA₁ was found not to change significantly in samples stored in this way.

The colorimetric method, based on that described by Fluckiger and Winterhalter,¹ was calibrated against fructose standards to read HbA₁ as mg fructose/g Hb. In our hands this method gave a mean ($\pm$ 2 SD) value of 1.9 ± 0.68 mg fructose/g Hb for normal subjects; the intra-assay coefficient of variation was 4.1%, the inter-assay coefficient of variation 7.6%, and the precision ($\sqrt{d^2/2n}$, where d = the difference between duplicate assays) 0.03. Using Biorad microcolumns the mean HbA₁ ($\pm$ 2 SD) for normal subjects was $7.7 \pm 1.70\%$ total haemoglobin, the intra-assay coefficient of variation 1.6%, the inter-assay coefficient of variation 2.9%, and the precision 0.26. The correlation coefficient for the two methods in this laboratory was 0.90.

In a group of 44 consecutive, newly diagnosed, untreated diabetics and 47 normal subjects we found a close correlation between total HbA₁ and both the fasting blood glucose concentration ($r=0.89$) and the area enclosed by the blood glucose curve ($r=0.85$) during a 50 g oral glucose tolerance test.

HbA₁ measured daily for five days in normal subjects varied very little. The mean values ($\pm$ SD) for each subject (expressed as mg fructose/g Hb) were: subject 1, 1.80 ± 0.07 ; subject 2, 1.81 ± 0.18 ; subject 3, 1.83 ± 0.21 ; subject 4, 1.86 ± 0.18 ; subject 5, 1.85 ± 0.12 .

Results

HbA₁ AND 24-HOUR URINARY GLUCOSE

Measurement of the 24-hour urinary glucose excretion was used as the main index of overall control of blood glucose, supplemented by measurement of fasting and postprandial blood glucose concentrations. These two indices of diabetic control were closely related in all patients studied. For clarity only the values for 24-hour urinary glucose and the corresponding HbA₁ concentration on the morning of completing the collection are plotted for individual patients in the figure.

Four patients (cases 1, 2, 5, and 7) had an increase in HbA₁ concentrations of 20% or more (table II) in the fourth or fifth week of the

study. The peak occurred within 10 days of reducing the dose of insulin and coincided with the period of heaviest glycosuria. There was a secondary, much smaller, rise in HbA₁ concentration of doubtful significance (12-17%) in the eighth or ninth week of the study—about five to six weeks after the period of poor control—cases 1, 2, and 5. Control was not optimal in case 2 in the follow-up period, but was considerably better than in the period of poor control. Unfortunately, follow-up was limited to three weeks in case 7.

In contrast, two patients (cases 3 and 6) showed no acute rise in HbA₁ concentration despite heavy glycosuria when their insulin was reduced. One (case 6) showed a significant rise (35%) in HbA₁ concentration in the 11th week of the study—that is, eight weeks after the period of poor control; the other patient (case 3) showed no change whatsoever in HbA₁ throughout the study despite the acute metabolic decompensation for 10 days. Case 4 showed minor and insignificant fluctuations in HbA₁ concentration during the fourth and fifth weeks of the study when the reduced insulin dose resulted in only a minimal increase in glycosuria. There was no secondary peak.

There was no significant difference in the pattern of change in HbA₁ concentration in individual patients when it was estimated by colorimetric and chromatographic methods (see figure, cases 1 and 3).

These patterns of change in HbA₁ concentrations in individual patients are summarised in table II and related to changes in diabetic control.

OTHER VALUES

Fasting blood glucose—The mean fasting and postprandial blood glucose concentrations for each patient are shown in table III.

Plasma urea and electrolytes were measured twice daily during the period of poor control to monitor the degree of metabolic decompensation. No changes sufficient to warrant terminating the study were observed (table II).

Serum lipids and lipoproteins—The fasting serum concentrations of cholesterol, triglycerides, and phospholipids showed no significant trend or pattern throughout the study.

Discussion

HbA_{1c} (the principal component of HbA₁) is thought to be formed by condensation of glucose with the N-terminal amino-groups of the β chain of HbA by a relatively slow, probably non-enzymatic, process continuing throughout the life span of the erythrocyte and its rate of synthesis seems to be a function of the blood glucose concentration.²

An early study³ showed that there was no correlation between concentration of HbA_{1c} (measured by macrocolumn

24-hour urinary glucose excretion and glycosylated HbA₁ concentrations during study in each of the seven patients. HbA₁ values shown are those measured by colorimetric method except in cases 1 and 3, where values measured by microcolumn method are also shown (broken line).
Conversion: SI to traditional units—Glucose 1 mmol/24 h ≈ 0.18g/24 h.

TABLE III—Mean (± SEM) fasting blood glucose concentrations (mmol/l) in initial assessment period and on days 3-10 of inpatient study and postprandial concentrations on days 3-10 of inpatient study.

Case No.	1	2	3	4	5	6	7
Fasting blood glucose:							
Assessment period	11.5 ± 0.69	7.1 ± 0.40	9.1 ± 3.2	9.6 ± 1.71	2.2 ± 0.23	7.7 ± 1.08	7.7 ± 0.53
Inpatient study (days 3-10)	15.5 ± 1.06	8.3 ± 0.41	15.9 ± 1.98	5.2 ± 0.23	18.0 ± 0.71	11.4 ± 0.65	14.0 ± 1.67
Postprandial blood glucose	10.9 ± 0.86	9.7 ± 0.80	17.4 ± 2.12	7.6 ± 1.09	18.6 ± 0.87	11.8 ± 2.06	12.9 ± 1.61

chromatography) and the type of diabetes, duration of disease, age, or presence of complications. Other studies have variously shown good statistical correlation between the concentration of HbA_{1c} (measured by macrocolumn chromatography) and the fasting and peak blood glucose concentration during an oral glucose tolerance test,⁴ the mean blood glucose concentration immediately before and one hour after the three meals of a day,⁵ and the mean of several daily plasma glucose values in diabetics being treated in various ways.⁶

In another study⁷ the relationship between the concentration of HbA_{1c} (measured by an electrofocusing method) and various indices of blood glucose control obtained over the previous eight weeks was examined. HbA_{1c} concentrations correlated with neither the mean fasting or postprandial blood glucose concentration nor the 24-hour urinary glucose but they did correlate with semiquantitative preprandial urine analysis.

The time relation between changes in HbA_{1c} concentration and in glucose tolerance and diabetic control has not been clearly defined. Koenig *et al*⁵ stated that a fall in HbA_{1c} concentration may lag three to four weeks behind improved diabetic control as judged by diminished urinary glucose excretion. Paulsen and Koury⁸ found that HbA_{1c} concentrations were high in patients with diabetic ketoacidosis and that after treatment the concentration fell slowly, taking at least a month to reach the level commonly found in non-ketotic diabetics. Ditzel and Kjaergaard⁹ studied the response of HbA₁ concentrations to treatment of newly diagnosed diabetic patients and found that it took 25 to 80 days to approach normal, depending on the initial HbA₁ concentration and the blood glucose control achieved. Brooks *et al*¹⁰ found considerable variation in individual patterns of response of HbA₁ concentrations to treatment of the diabetes, which could not obviously be accounted for by differences in the overall response of the blood glucose. The observations of Leslie *et al*¹¹ indicated that "a single HbA₁ estimation may relate to a shorter period of hyperglycaemia than has been suggested." Svendsen *et al*^{12, 13} have provided evidence from studies conducted both *in vivo* and *in vitro* that a significant proportion of HbA₁ is formed by a fast and rapidly reversible process. This is consistent with a saturable system of glycosylation as suggested by Graf *et al*.¹⁴

The fact that four of our seven patients had an acute rise and fall in HbA₁ concentrations during the period of poor control, which was more impressive than any later rise, supports the concept that fast and rapidly reversible glycosylation can occur in at least some insulin-dependent diabetic patients. In contrast, two other patients with very significant hyperglycaemia and glycosuria during the period of poor control showed no acute rise in HbA₁ concentrations. There was no obvious difference between these two groups of patients to account for their different patterns of HbA₁ response. We are confident that these varied responses are real and not simply due to methodological error, since two methods of estimation, which use different principles of measurement, showed similar patterns of HbA₁ response in individual patients.

Our studies in newly diagnosed, untreated diabetic patients (unpublished) confirm that in general the rate of synthesis of HbA₁ is a function of the blood glucose concentration—that is, in groups of such patients the relationship between blood glucose and HbA₁ concentrations holds. Nevertheless, the considerable individual variation, shown in the response of HbA₁ to treatment of diabetes¹⁰ and here to a period of poor control, may diminish the clinical usefulness of HbA₁ concentrations as a monitor of overall diabetic control in individual diabetics. This may apply particularly to the insulin-dependent diabetic, in whom rapid and short-term fluctuations in blood glucose concentration more commonly occur, and we suggest that single values for HbA₁ should be interpreted with considerable caution in these patients.

More detailed studies are required in insulin-independent patients and more information is needed about the sensitivity of HbA₁ concentrations in detecting periods of poor control. The fact that only one patient (case 6) showed a significant rise in

HbA₁ concentrations in the two months of observation after the period of poor control suggests that HbA₁ may be a relatively insensitive method of detecting short periods of poor diabetic control.

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ONE HUNDRED YEARS AGO We regret to learn that the Local Government Board have found it necessary to call on Mr R Bruce, the Medical Superintendent of the Sick Asylum, Upper Holloway, belonging to the Holborn Union, to send in his resignation; and the regret we entertain is not lessened on perusal of the letter addressed to that gentleman by the Board. It would appear that a patient, of the name of Stapleton, was admitted into the infirmary suffering from congestion of the lungs and delirium. Unfortunately he was not seen on admission by Mr Bruce, but only by the assistant medical officer, and in the course of that night he was put into a bath, without any order for the same by the medical officer, by two pauper attendants. Subsequently he was sent, under the direction of Mr Bruce, we presume on account of his mental excitement, to the Gray's Inn Road Workhouse, a distance of about four miles, where he shortly afterwards died; and on *post mortem* examination, it was found that the cause of death was inflammation of the lungs. There were some other charges of want of carefulness, and of refusal to admit cases to the asylum, preferred against Mr Bruce; and these, together with Stapleton's, above quoted, led to the Board peremptorily demanding his resignation. We would earnestly point out to gentlemen holding poor-law appointments, that they cannot be too particular in examining patients as to their fitness to bear a journey ere they direct their removal from a district to a workhouse, or from this latter to a hospital, lunatic, or sick asylum; for, if the patient should happen to die shortly after such removal, the medical officer would be apt to incur much blame. And it is not merely for their own sake, but for the credit of the profession to which they belong, that the utmost circumspection is necessary on the part of all gentlemen holding these appointments. (*British Medical Journal*, 1880.)