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Evidence of hypoxaemia and distribution of minor haemoglobin components in the cord blood of neonates born to diabetic mothers

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Summary

The oxygenation status of normal and diabetic (White's classification A and B) mothers and their neonates was investigated. The diabetic patients had significantly increased maternal total haemoglobin and P_{50} values and the percentage of fetal haemoglobin was increased in cord blood taken at delivery in this group. There was a significant positive correlation between maternal P_{50} values and the percentage of fetal haemoglobin in cord blood. The cord blood 2,3-diphosphoglycerate, inorganic phosphate and P_{50} values were also increased in neonates born to diabetic mothers and these infants had a significantly increased birth weight ratio. The results are consistent with the presence of fetal hypoxaemia in the late third trimester of diabetic pregnancy in which obvious maternal vascular disease has been excluded.

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Diabetic pregnancy is associated with a variety of complications which include fetal macrosomia and increased risk of intra-uterine death.¹ Macrosomic infants *per se* show increased mortality and morbidity.²

In a study of poorly controlled insulin-dependent pregnant diabetics (as indicated by increased levels of Hb A_{1c}) Madsen and Ditzel³ presented evidence of impaired oxygen transport/delivery to the tissues. They concluded that this hypoxaemia was unlikely to be due solely to the increased levels of Hb A_{1c} (since Hb A_{1c} has an increased affinity for oxygen), but was due instead to multiple factors, including structural placental abnormalities in these patients. As early as 1954 the presence of fetal hypoxia in human diabetic pregnancy was suggested because of increased cord blood erythropoietin,⁴ and more recently this hypothesis has been confirmed by Widness *et al*.⁵ Experiments performed after tolbutamide⁶ or insulin infusion^{7,8} into chronically catheterized fetal lambs have attributed this hypoxaemia to hyperinsulinaemia and it is possible that hypoxaemia may play a role in the increased mortality and morbidity associated with diabetic pregnancy.⁷

Investigations into the possible occurrence of cord blood hypoxaemia in pregnant diabetics without vascular complications and what factors were involved were carried out.

Patients and methods

Patients

Twenty-nine pregnant diabetic women and 20 pregnant non-diabetic controls were studied. The patients were predominantly of the Cape Coloured race group, but included 5 whites and 1 Indian (White's⁹ class B). The non-diabetic

controls had normal glucose tolerance tests according to the criteria of O'Sullivan and Mahan.¹⁰ Only pregnant diabetics of White's⁹ class A (18 patients) and B (11 patients) were included in the study. (Patients with vascular complications were carefully excluded.) Gestational diabetics (class A) were treated with a standard diabetic diet¹¹ and after initial hospitalization, during which daily plasma glucose profiles confirmed adequate control, they were observed in hospital for 1 day every week. The aim of the dietary therapy was to keep the 2-hour postprandial plasma glucose value below 8,3 mmol/l. Class B diabetics had fasting plasma glucose levels above 5,8 mmol/l and appropriate insulin therapy was aimed at maintaining this at below 5,8 mmol/l and the 2-hour postprandial value at below 8,3 mmol/l. Treatment of class B diabetics was conducted on an inpatient basis almost throughout pregnancy.

According to the above criteria all patients were considered to be well controlled. Intrapartum euglycaemia was controlled by frequent estimations of plasma glucose. The maternal age distribution showed that both class A ($30,6 \pm 7,2$ years) and class B ($33 \pm 5,2$ years) diabetic patients were older than the non-diabetic controls ($26,1 \pm 5,7$ years) ($P < 0,05$ and $P < 0,01$ respectively).

Forty-five of the infants born to the mothers described were studied. No infants were born before 36 weeks' gestation and those of the diabetic patients showed no significant difference from controls in gestational age at birth ($38,6 \pm 1,2$ weeks and $38,9 \pm 0,84$ weeks respectively). All the infants studied were born by normal uncomplicated vaginal delivery. Mothers with problems during labour (e.g. prolonged second stage, cord compression) or who received any form of anaesthesia, were excluded. Gestational age was determined postnatally using the Dubowitz estimation.¹² The relative birth weight ratio (RBWR) was then calculated by dividing the actual birth weight by the 50th percentile for gestational age using percentile charts appropriate for the local population.¹³

Informed consent was obtained from all patients. Maternal venous blood specimens were collected on the day of delivery and cord blood was collected at delivery (before the infant's first breath) directly from the umbilical vein, as described by Prystowsky *et al.*¹⁴

Methods

Maternal haemoglobin (Hb A) was measured by means of a Coulter Counter model S-Plus (Coulter Electronics Inc., Hialeah, Florida, USA) and the results expressed as g/dl.

The percentage of total glycosylated haemoglobin (% Hb A₁) was measured on venous blood collected into ethylenediamine tetra-acetic acid (EDTA)-containing tubes which were then stored at 4°C until assayed (always within a maximum of 3 days). A commercial kit utilizing an ion-exchange microcolumn chromatography procedure was used (Diagnostic Corp., Arlington, Texas).

For the determination of the percentage of Hb F₀ (the main component of fetal haemoglobin in the cord blood) and the other minor components in the umbilical vein, blood was collected into EDTA-containing tubes and a chromatographic procedure¹⁵ was used to separate the components. The red blood cells obtained by centrifugation (3000 rpm for 10 minutes) were washed three times by further centrifugation with 0,15M NaCl and lysed with an equal volume of haemolysing solution (1% saponin in buffer A (15 g glycine + 0,1 g KCN per litre at a final pH of 7,8)). The lysate was diluted 1:1 with buffer A and dialysed overnight with stirring against 1:1 buffer A/water at 4°C. The dialysates (100 - 150 µl) were separated on a Whatman DE 52 cellulose column (pH 7,8) using an ionic strength gradient from 0,02M NaCl (150 ml) to 0,04M NaCl (150 ml). The flow rate was 24 ml/h, the fraction

size 3,0 ml and the column dimensions 25 x 1,0 cm. A typical elution profile with details of the chromatographic separation is shown in Fig. 1. The total area under all of the eluted peaks was calculated and the amount of each component peak was expressed as a percentage of the total area. Identification of the eluted peaks was performed according to Abraham *et al.*¹⁵ and Schwartz *et al.*¹⁶ Separate tracer experiments to identify the individual peaks were not carried out. The Hb F_{1a} peak has been designated as glycosylated fetal haemoglobin and the Hb F₁ peak as acetylated fetal haemoglobin (Fig. 1).

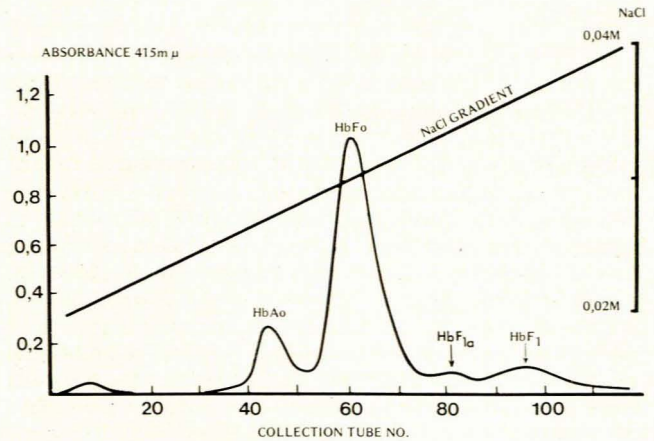


Fig. 1. A typical chromatographic elution profile showing Hb F₀ (the main component of fetal haemoglobin), Hb A₀ (the percentage of adult haemoglobin in the cord blood), Hb F₁ (the acetylated fetal haemoglobin), and Hb F_{1a} ((putative) glycosylated fetal haemoglobin).^{15,16}

A quantitative procedure (Sigma Chemical Co., St. Louis, Mo., Kit No. 35-UV), which incorporates sequential enzyme systems linked to the production of nicotinic acid dehydrogenase, was used to determine 2,3-diphosphoglycerate (2,3-DPG). For this purpose umbilical vein blood was collected into heparin-containing tubes at delivery. Deproteinization was then carried out by using ice-cold trichloro-acetic acid as described by Gordon-Smith¹⁷ and the clear supernatant was stored at 4°C until the determination of 2,3-DPG was carried out (within 3 weeks).

The estimation of P₅₀ values (partial arterial pressure of oxygen (PaO₂) at which haemoglobin is 50% saturated with oxygen) was done on 10 ml of maternal venous blood collected into heparinized syringes which were immediately sealed. Care was taken to ensure that no air bubbles were present in the blood. To avoid blood stasis after inserting the needle into the mother's vein the pressure of the tourniquet was released for a few minutes before the blood was drawn. The same amount of cord blood was collected into heparinized syringes after the needle was inserted directly into the umbilical vein and the syringes were immediately sealed.

The measurement of pH, partial arterial carbon dioxide pressure (PaCO₂) and base excess was determined immediately on an Instrumentation Laboratory (IL) (Lexington, Mass.) blood gas analyser model 613 and the measurement of haemoglobin, percentage oxygen saturation (% SO₂) and percentage carboxyhaemoglobin (% HbCO) was done on an IL co-oximeter model 282. The P₅₀ value was then calculated from a single measurement of pH, PaO₂ and % SO₂, as described by Aberman *et al.*,¹⁸ with correction for the amount of HbCO present in the blood samples, as described by Ledwith.¹⁹ In this way the P₅₀ value at pH 7,40 with the temperature at 37°C was obtained.

Inorganic phosphate (P_i) estimation of umbilical vein blood collected into heparin-containing tubes was carried out the

same day using a Technicon SMAC Analyzer. Care was taken to exclude haemolysed specimens.

Statistical analysis was carried out using parametric (Student's *t*-test and linear regression analysis with Pearson's correlation coefficient (*r*)) as well as non-parametric methods (Mann-Whitney *U*-test and Spearman's rank correlation coefficient (*r_s*). The *P* and the *r* values (two-tailed test) refer to non-parametric statistics, unless otherwise indicated. All *P* values of < 0,05 were accepted as statistically significant.

Results

The Hb A and the P₅₀ values of the diabetic mothers were significantly higher than those of the normal mothers (Table I). However, the percentage of Hb A₁ did not differ significantly in the two groups. (The range for normal non-pregnant subjects in this study was 5,5 - 8,5%.) The RBWR and the P_i levels of the infants born to diabetic mothers (IDMs) were also significantly higher than those present in infants born to normal mothers (INMs). In addition the IDMs were found to have a significantly higher percentage of Hb F₀ than that present in INMs (Table I). There was no significant difference in gestational age in the two groups of neonates and the Hb F₀ values were plotted in relation to their respective gestational age. The downward trend was then fitted and the deviation taken from this trend. The difference between the two groups of neonates was significant at *P* < 0,02 (not shown). The mean percentage of Hb F₁ in IDMs was 7,9 ± 1,6% and this value did not differ significantly (*P* > 0,1) from that of INMs (7,1 ± 1,5%). A small Hb F_{1a} peak was detected in the cord blood of only 4 infants: 1 in the group of INMs with 4% Hb F_{1a} and 3 in the group of IDMs with concentrations of Hb F_{1a} of less than 2%.

There was a highly significant inverse correlation between neonatal P₅₀ values and the percentage of Hb F₀ in the INMs (Fig. 2A). In contrast, no such correlation was found in IDMs (Fig. 2B). The P₅₀ value of the IDMs was significantly higher than that of INMs (Table I).

The IDMs had higher 2,3-DPG levels than the INMs (Table I) and in addition a significant positive correlation between P₅₀ values and 2,3-DPG levels was found in the IDMs (Fig. 3B). This relationship was absent in the INMs (Fig. 3A).

Fig. 4 illustrates the correlation between P₅₀ values of normal (A) and diabetic (B) mothers and the percentage of Hb F₀ in their infants. There was no significant correlation in the normal mothers, while a significant positive correlation was found in the diabetic mothers.

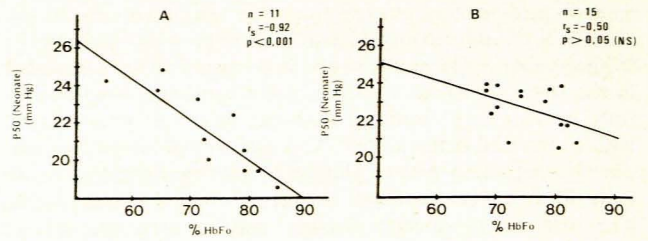


Fig. 2. Correlation between P₅₀ values and the percentage of Hb F₀ in neonates born to normal (A) and diabetic (B) mothers.

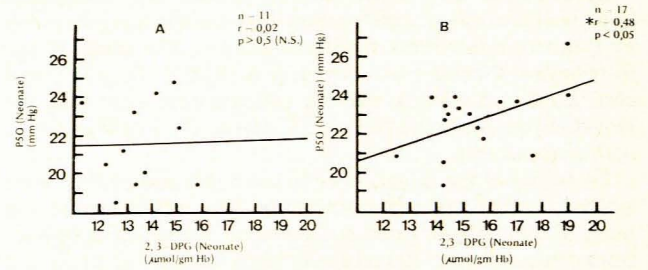


Fig. 3. Correlation between P₅₀ values and 2,3-DPG levels in neonates of normal (A) and diabetic (B) mothers (* the *P* value refers to parametric statistics as the 2,3-DPG values have a Gaussian distribution). Although it seems that the regression line in Fig. 3A is greatly conditioned by the single point near the vertical axis with 2,3-DPG value 11,2 μmol/g Hb, omission of this point again produced a non-significant correlation (*r* = 0,27); however, no clinical grounds were found for excluding this patient.

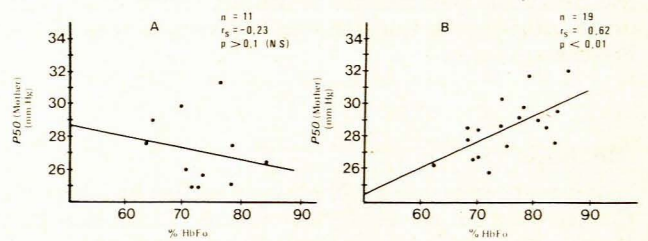


Fig. 4. Correlation between maternal P₅₀ values of normal (A) and diabetic (B) mothers and the percentage of Hb F₀ in their neonates.

TABLE I. DATA FOR THE MOTHERS AND THEIR INFANTS (MEAN ± SD)

	Mothers		Infants	
	Normal (N = 20)	Diabetic (N = 29)	iNM (N = 17)	IDM (N = 28)
Hb A (g/100 ml)	12,4 ± 1,4	13,2 ± 1,2†	14,6 ± 1,2	15,6 ± 1,8†
Hb A ₁ (% total Hb A)	6,8 ± 0,7	7,2 ± 1,2*	—	—
RBWR	—	—	1,04 ± 0,1	1,15 ± 0,15†
Hb F ₀ (% total Hb in cord blood)	—	—	69,9 ± 9,2	76,9 ± 6,4†
P ₅₀ (mmHg)	26,9 ± 1,92	28,9 ± 1,6‡	20,86 ± 1,83	22,49 ± 1,38‡
2,3-DPG (μmol/g Hb)	16,2 ± 1,6	16,75 ± 1,5*	14,0 ± 1,4	15,4 ± 1,7†
Phosphate (mmol/l)	1,03 ± 0,2	1,13 ± 0,19*	1,49 ± 0,15	1,78 ± 0,42‡

*Not significant.
† *P* < 0,05.
‡ *P* < 0,02.

Discussion

According to the criteria for this study (see 'Patients') all the diabetic mothers were well controlled and this was confirmed by the absence of any significant increase in Hb A₁ in these patients (Table I).

Studies on *in vitro* synthesis of Hb F₀ and Hb A₀ in neonatal reticulocytes²⁰ and in the liver of a 17-week fetus²¹ have shown that under hypoxic conditions an increased proportion of Hb F₀ is synthesized. Thus the significantly increased percentage of Hb F₀ observed in the IDMs (Table I) suggests, in the absence of any significant difference in gestational age between the two groups, that fetal hypoxia was present in these infants. Increased levels of fetal Hb A and even real polycythaemia are known to occur in IDMs as a result of maternal diabetic angiopathy.² This is unlikely to explain the results of this study since mothers with vascular disease were carefully excluded. In addition, infants born to diabetic mothers with advanced vascular disease and placental abnormalities are small for gestational age,¹⁰ while the IDMs in this study had significantly higher RBWRs than those of INMs (Table I).

The increased Hb A and P₅₀ values found in the diabetic mothers (Table I) as well as the significant correlation between the P₅₀ values in diabetic mothers and the percentage of Hb F₀ in their neonates (Fig. 4B) further supports the presence of an increased fetal oxygen demand in diabetic pregnancy and an attempt by the diabetic mother to compensate for this by releasing more oxygen to the placental tissue and thus to the fetus.

The highly significant ($r_s = 0.92$; $P < 0.001$) negative correlation between neonatal P₅₀ values and the percentage of Hb F₀ in INMs (Fig. 2A) indicates that the percentage of Hb F₀ is the major factor determining the position of the oxygen dissociation curve (ODC) in the INMs, a finding which agrees with Riegel *et al.*²² but differs from Orzalesi and Hay's²³ suggestion that the relative proportions of fetal and adult haemoglobin and the 2,3-DPG levels determine the position of the normal neonatal ODC. However, the absence of such a correlation in the IDMs (Fig. 2B) suggests that the percentage of Hb F₀ does not influence the ODC of IDMs to the same extent as in INMs. Moreover, results in this study showed that the IDMs have significantly higher P₅₀ values than those present in the INMs (Table I) in spite of the fact that IDMs have increased levels of Hb F₀ (Table I).

The fetal erythrocyte seems to be uniquely designed to survive in a hypoxic environment,²⁴ and this can be seen from the normal fetal ODC which is 'left-shifted' (P₅₀ at term approximately 20 mmHg) compared to the normal adult ODC (P₅₀ approximately 26 - 27 mmHg).²⁵⁻²⁷ This left shift is also generally believed to facilitate oxygen transport across the placenta.²⁸ A right shift of the fetal ODC is not beneficial to the fetus since less oxygen will be delivered to the fetal tissues,^{28,29} and the presence of increased P₅₀ values suggests a hypoxic situation in the IDMs. (In contrast, a similar right shift of the adult ODC is beneficial because more oxygen is delivered to the adult tissues.³⁰)

The 2,3-DPG is known to influence the adult ODC significantly (i.e. an increase in 2,3-DPG causes a right shift), but it is thought to interact with the fetal haemoglobin to a lesser extent. This is generally accepted as the primary reason for the left shift in the fetal ODC.³¹ Nevertheless, this study found that IDMs not only had significantly higher 2,3-DPG levels than those of INMs (Table I), but in addition these levels correlated well with the neonatal P₅₀ values (Fig. 3B). Such a correlation was not present in INMs (Fig. 3A). This suggests that the increased 2,3-DPG levels found in the IDMs contribute to the right shift of the ODC found in these infants. This correlation was somewhat unexpected. It is well known that in adults the presence of mild hypoxia leads to an increase

in 2,3-DPG levels,^{32,33} although *in vitro* experiments with erythrocytes of newborns from normal mothers suggested that this may not be the case in neonates.³⁴ The results of this study suggest that a similar mechanism to that of the adult may operate in IDMs. This response is, as already discussed, inappropriate, since it reduces the oxygen delivery to the fetal tissues^{28,29} and may therefore aggravate the hypoxic condition of these infants.

The plasma pH and P_i are known to be important factors controlling adult P₅₀ values and 2,3-DPG levels.^{30,35-37} The plasma pH of the IDMs was similar to that of INMs (not shown) while the P_i levels were significantly increased (Table I), but the levels did not correlate with the 2,3-DPG values ($r = 0.1$; $P < 0.5$, not shown). Nevertheless, it is felt that a possible combination of all the above factors (i.e. hypoxia with increased deoxy-Hb F₀ and hyperphosphataemia) could lead to an *in vivo* increase in 2,3-DPG levels in IDMs, and could explain the difference in cord blood P₅₀ values in the two groups of patients.

The cause of the hypoxia present in the IDMs is unclear, and there is little direct evidence for fetal hypoxia in diabetic pregnancy in the absence of advanced vascular disease.^{4,5,38} For this reason the term 'relative' hypoxia has been used to describe the hypoxic state in IDMs, although the phrase 'tendency to hypoxaemia' may be more accurate. Recent experiments in fetal lambs⁶⁻⁸ have shown that sustained hyperinsulinaemia produces profound hypoxia in these fetuses, and it has been suggested⁶ that this hypoxia may play a role in the increased risk of intra-uterine death in diabetic pregnancy. Since fetal hyperinsulinism has been demonstrated in neonates from diabetic mothers³⁹ and this hyperinsulinism correlated with the macrosomia and postnatal hyperglycaemia found in these infants,³⁹ it is tempting to speculate that the relative hypoxia present in the IDMs could be a result of hyperinsulinism and also that the presence of relative hypoxia could be related to the fetal macrosomia which commonly occurs in diabetic pregnancy. This latter suggestion is supported by the finding of increased RBWR in our IDMs (Table I), but it must be stated that we could find no significant increase in maternal Hb A₁ values in our diabetic mothers. Nevertheless macrosomia in neonates from well-controlled diabetic pregnancies has been documented recently,⁴⁰⁻⁴³ and good diabetic control of the mother does not exclude the possibility of hyperinsulinism in the fetus.

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Nuus en Kommentaar/News and Comment

Parachuting and neck injury

It takes a particular form of single-mindedness to want to jump out of a perfectly serviceable aeroplane. However, free-fall parachuting has become a popular leisure-time activity, and musculoskeletal injuries (apart from those caused by the parachute failing to open) can be expected to increase in frequency. Not all of the injuries caused by parachuting may be obvious at first sight. A case is described of a 24-year-old parachuting instructor with 400 successful descents who developed hyperaesthesia of the right arm and mild inco-ordination of the right leg following a jump (*Injury* 1984; **16**: 9). As radiographs and laboratory investigations were normal at the time, a demyelinating disease was suspected, and he was treated with adrenocorticotrophic hormone following which he improved slowly. Nine months later, after a further episode of free-fall parachuting, he developed transient paraesthesiae in all limbs and a flaccid tetraparesis lasting a few seconds. The

paraesthesiae improved over the next 3 hours but a mild occipital headache persisted. A month later, after a similar descent, he developed severe paraesthesiae and a complete tetraplegia. At this stage, a plain radiograph of his cervical spine showed spina bifida occulta of the atlas and posterior osteophytes on the C5/C6 interspace. However, cervical myelography demonstrated a large C5/C6 disc protrusion partially obstructing the spinal canal.

The opening shock as a parachute deploys may place considerable stress on the cervical spine and can produce forced flexion injuries affecting the spinal cord, particularly if a heavy helmet is worn. In this case, the parachutist also had a camera attached to the helmet. Parachute instructors are insistent on correct posture of the head and neck when deploying the parachute. It would appear that they are absolutely right to do so.