SHORT REPORTS

Glycosylated fetal haemoglobin

Glycaemic control in the last trimester of pregnancy may be assessed by measuring the amount of glycosylated haemoglobin (HbA_{1c}) in maternal blood. This has been shown to be related to the neonatal skinfold thickness, which currently appears to be the best method for determining the impact of diabetic control during pregnancy on the fetus.¹ A more direct way of assessing fetal glucose concentrations during late pregnancy might be to measure glycosylated haemoglobin in fetal blood. The stepwise-elution ion-exchange methods usually used do not, however, differentiate between glycosylated adult haemoglobin (HbA) and fetal haemoglobin (HbF). We have used an isoelectric focusing (IEF) method to investigate the relation between the glycosylated haemoglobin fractions of the mothers and their babies.

Subjects, methods, and results

Maternal venous and fetal cord blood samples were obtained simultaneously from 10 insulin-treated and 14 non-diabetic mothers and their babies immediately after delivery. All blood samples were diluted with saline (1:10 v/v) and were kept at 4°C. Potassium cyanide haemolysates were prepared for the separation of the haemoglobins by the IEF technique.² Cord blood from four normal babies was also incubated with 100 mmol/l (1.8 g/100 ml) glucose at 37°C for 2 hours. The bands representing HbF, HbA, HbA_{1c}, and glycosylated fetal haemoglobin (HbF₁) were excised from the electrophoretic gels and eluted for measurement with a spectrophotometer.

On IEF separation of the cord blood from non-diabetic mothers, three discrete bands could be seen: HbF (mean $\pm$ SD) $67.1 \pm 8.1\%$; HbA $23.0 \pm 6.2\%$; and HbF₁ $7.8 \pm 1.5\%$, previously reported to be acetylated fetal haemoglobin.³ The values for the babies of diabetic mothers were similar: HbF $63.0 \pm 9.4\%$; HbA $18.4 \pm 3.9\%$; HbF₁ $8.6 \pm 1.9\%$; as were the maternal HbA_{1c} values from the non-diabetic and diabetic mothers ($7.8 \pm 1.9\%$ and $8.0 \pm 2.0\%$ respectively). No HbA_{1c} could be observed, either visually or photometrically, in any cord-blood sample. When the cord blood was incubated with excess glucose, the amount of HbF₁ increased nearly twofold from 7.2 ± 1.1 to $13.9 \pm 2.3\%$, with a corresponding decrease (68.2 ± 4.1 to $57.0 \pm 3.7\%$) of HbF (see figure). The fetal HbF₁ of all cord-blood samples correlated with the simultaneous maternal HbA_{1c} (Spearman rank correlation $r_s = 0.4$, $p < 0.05$, $n = 24$). No correlation was observed between fetal HbF₁ or maternal HbA_{1c} and birth weight or percentile birth weight (corrected for gestational age, fetal sex, and maternal parity⁴).

In-vitro incubation of four cord blood samples at 37°C with 100 mmol/l (1.8 g/100 ml) D-glucose, showing a decrease in HbF and a simultaneous increase in HbF₁. Results of each case are shown together with the mean $\pm$ SD for each group.

Comment

There is relatively little HbA in cord blood, and its glycosylated form, HbA_{1c}, cannot be detected using the IEF technique. Since the major fraction of haemoglobin present in the newborn is HbF, and as glucose crosses the placenta freely by facilitated diffusion, it is

possible that HbF may be "glycosylated" non-enzymatically to form HbF₁ in a similar manner to the formation of HbA_{1c} in adults. Indeed, the in-vitro glucose incubation data and the correlation between HbA_{1c} and HbF₁ suggest this. Furthermore, it has recently been shown⁵ that non-diabetic alcoholics have raised HbA₁ concentrations and that acetaldehyde, produced from ethanol metabolism, also reacts with the N-terminus of the haemoglobin β -chain. Hence HbF₁ may be a combination of the acetylated and glycosylated moieties of HbF. Stepwise-elution chromatography and chemical methods—for example, Flückiger's method—cannot distinguish between fetal and adult haemoglobins, though they may reflect overall glycosylation. HbF₁ measured in cord blood by the IEF technique could be used as an index of fetal exposure to glucose during the last few weeks of pregnancy.

This investigation was funded by the Wellcome Trust. We wish to thank Mrs C Whittle for analysing the haemoglobin samples.

Addendum.—Since submission of this paper, Fadel *et al* (*Am J Obstet Gynecol* 1981;139:397) have reported similar results.

¹ Stubbs SM, Leslie RDG, John PN. Fetal macrosomia and maternal diabetic control in pregnancy. *Br Med J* 1981;282:439-40.

² Jeppsson J-O, Franzen B, Nilsson KO. Determination of the glycosylated haemoglobin fraction HbA_{1c} in diabetes mellitus by thin layer electro focussing. *Science Tools* 1978;25 (4):69-72.

³ Schroeder WA, Cua JT, Genji M, Fenniger W. Haemoglobin F₁, an acetyl-containing haemoglobin. *Biochim Biophys Acta* 1962;63:532-4.

⁴ Thomson AM, Billewicz WZ, Hytten FE. The assessment of fetal growth. *Br J Obstet Gynaecol* 1968;75:903-16.

⁵ Stevens VJ, Fantl WJ, Newman CB, Sims RV, Cerami A, Peterson C. Acetaldehyde adducts with haemoglobin. *J Clin Invest* 1981;67:361-9.

(Accepted 18 May 1981)

Diabetes Research Laboratories, Radcliffe Infirmary, Oxford OX2 6HE

P POON, BSC, ARCS, research biochemist
R C TURNER, MD, clinical reader

Nuffield Department of Obstetrics and Gynaecology, John Radcliffe Hospital, Oxford OX3 9DU

M D G GILLMER, MD, clinical reader

Arthritis of the subtalar joint due to *Mycobacterium fortuitum*

Mycobacterium fortuitum is known to cause infection¹ of the soft tissues but has rarely been implicated in joint infections.² We report a case of subtalar joint infection in which *M fortuitum* was repeatedly isolated from pus and curettings.

Case report

A 62-year-old Indian woman strained her right ankle in 1974. Pain and swelling persisted and three years later she had an operation in India for removal of osteophytes. After the operation radiography showed subtalar osteoarthritis. Three months after the operation she was in England and presented with continuing pain, wasted right peroneal muscles, and a stiff subtalar joint. Ten days later she developed a discharging sinus in the operation scar; radiography showed osteoporosis around the subtalar and ankle joints with partial erosion of the head of the talus. Culture of the pus yielded no growth, and she was given ampicillin and flucloxacillin. One month later there was also erosion of the midtarsal joint and the sinus was curetted. *M fortuitum* was isolated, resistant to rifampicin, ethionamide, aminosalicylic acid, isoniazid, streptomycin, ethambutol, thiacezone, capreomycin, and pyrazinamide. She was given isoniazid and rifampicin for one year with ethambutol for the first two months because in-vitro resistance patterns do not necessarily reflect the in-vivo response.

The disease progressed and three new sinuses appeared on the sole of her foot, *M fortuitum* being isolated from the pus. Biopsy specimens of the sinuses showed multiple non-caseating giant-cell granulomas. Three months later the discharge became more copious: *M fortuitum* was again grown, and

Pseudomonas aeruginosa was repeatedly isolated. The *P. aeruginosa* was treated with a 10-day course of gentamicin. Radical curettage of the subtalar joint was performed and during the next year the discharge stopped and radiography showed diminution of cavity size with ankylosis. She subsequently walked well.

Intradermal skin tests for *M. fortuitum* were performed using ranin 1, 2, and 4 and chelonin, which are antigens specific for *M. fortuitum* serotypes 1, 2, and 4 and *M. chelonae* respectively. A 0.1 ml volume of each was injected, and after 72 hours she had areas of induration 6 mm in diameter at the site of the serotype 4 injection but less than 3 mm at the *M. chelonae* site, and no response at the other two sites. Induration of 5 mm or more is considered to be positive.

Comment

M. fortuitum is the commonest of the Runyon group IV rapidly growing mycobacteria. It may cause pulmonary, lymph-gland, soft-tissue, corneal, heart-valve, and prostatic infections.¹ In bone vertebral abscesses, medial sternotomy infections, and osteomyelitis of a phalanx after a scraping and of a calcaneum after a puncture wound have been reported. Ohry *et al.*³ described a case in which *M. fortuitum* osteomyelitis occurred after major trauma with open fractures. Wegmann *et al.*² reported the case of a woman who had suffered from peritoneal and cervical tuberculosis, poliomyelitis, hepatitis, and ankylosing spondylitis: she had received several cortisone injections to her right shoulder over some years. She presented with *M. fortuitum* arthritis in her right shoulder and ankle.

Antibiotic resistance is always a problem with *M. fortuitum*. The standard drugs for *M. tuberculosis* are rarely useful. Ethionamide, capreomycin, cycloserine, and the aminoglycosides⁴ may occasionally be useful. In our case there was borderline sensitivity to cycloserine and also to kanamycin, so possibly the gentamicin, given for pseudomonas infection, may have helped to eliminate the *M. fortuitum*. In a review of published reports Halpern and Nagel⁵ concluded that incision and debridement are the mainstay of effective treatment. We assume that the infection in our case was due to contamination of an arthritic joint at the time of the first operation.

We thank Dr P A Jenkins, Mycobacterium Reference Unit, Cardiff, for identifying the organism and sensitivity testing, and Dr J L Stanford, Middlesex Hospital, who supplied the intradermal antigens.

¹ Wolinsky E. Nontuberculous mycobacteria and associated diseases. *Am Rev Respir Dis* 1979;119:107-159.

² Wegmann T, Jutz P, Pagon S. Atypische Mykobakteriose, hervorgerufen durch *Mycobacterium fortuitum*. *Schweiz Med Wochenschr* 1978;108:684-6.

³ Ohry A, Brooks M, Steinbach T, Rozin R. Osteomyelitis caused by *Mycobacterium fortuitum*. *Infection* 1977;5:46-8.

⁴ Nicholson DP, Savier WR. *Mycobacterium fortuitum* as a pathogen. A case report. *Am Rev Respir Dis* 1971;104:747-50.

⁵ Halpern AA, Nagel DA. *Mycobacterium fortuitum* infections: a review with two illustrative cases. *Clin Orthop* 1978;136:247-52.

(Accepted 20 May 1981)

Whipps Cross Hospital, London E11 1NR

G B COLVER, MRCP, registrar in chest medicine
B CHATTOPADHYAY, MRCPATH, DCP, director, consultant microbiologist
R S FRANCIS, MD, FRCP, consultant chest physician
K M N KUNZRU, FRCS, consultant orthopaedic surgeon

Prostacyclin infusion in haemolytic-uraemic syndrome of children

Pathological intravascular platelet aggregation plays a major role in the pathogenesis of the microangiopathy of the haemolytic-uraemic syndrome and thrombotic thrombocytopenic purpura. Deficiency of endogenous prostacyclin (PGI₂), a potent inhibitor of platelet aggregation, has been shown in both conditions,^{1,2} and this could favour the formation of platelet thrombi in the microcirculation. PGI₂ replacement seems a logical therapeutic manoeuvre, but results in adults have been variable.¹⁻³ We report our experience with PGI₂ infusion in three children with the haemolytic-uraemic syndrome.

Case reports

Case 1—An 18-month-old girl was admitted after a diarrhoeal illness. On admission the blood pressure was 90/50 mm Hg, she was oliguric (20 ml/day), serum creatinine concentration was 400 µmol/l (4.52 mg/100 ml), platelet count was 25 × 10⁹/l, serum concentration of fibrin degradation products (FDP) was 80 mg/l, and burr cells were present on the peripheral blood film. Peritoneal dialysis was carried out for seven days. On the sixth day in hospital PGI₂ infusion was started at an initial dose of 2.5 ng/kg/min and increased to 30 ng/kg/min within nine days. The total duration of the infusion was 12 days. Diuresis started on the 15th day in hospital, with progressive improvement in renal function. The platelet count rose to 150 × 10⁹/l by the 14th day and the serum FDP concentration fell to normal by the 19th day. Five weeks after admission both blood pressure and renal function were normal.

Case 2—A 2-year-old girl was admitted after a diarrhoeal illness. On admission the blood pressure was 120/70 mm Hg, she was anuric, serum creatinine concentration was 520 µmol/l (5.87 mg/100 ml), platelet count was 120 × 10⁹/l, serum FDP concentration was 160 mg/l, and burr cells were present on the peripheral blood film. Peritoneal dialysis was carried out for 10 days. On the fourth day in hospital PGI₂ infusion was started at an initial dose of 2.5 ng/kg/min, increasing to 40 ng/kg/min within seven days. The total duration of the infusion was 10.5 days. Diuresis began on the 14th day with progressive improvement in renal function. The platelet count rose to 300 × 10⁹/l and the serum FDP concentration fell to normal by the 16th day. Ten weeks after admission the blood pressure was normal and the ⁵¹Cr-EDTA clearance was 69 ml/min/1.73 m².

Case 3—A 5-year-old girl was admitted after a diarrhoeal illness. On admission the blood pressure was 125/85 mm Hg, she was oliguric (10 ml/day), serum creatinine concentration was 540 µmol/l (6.1 mg/dl), platelet count was 25 × 10⁹/l, serum FDP concentration was 20 mg/l, and burr cells were present on peripheral blood film. Peritoneal dialysis was carried out for seven days. On the second day in hospital PGI₂ infusion was started at an initial dose of 2.5 ng/kg/min, increasing to 55 ng/kg/min within 12 hours. The total duration of the infusion was eight days. Diuresis began on the seventh day in hospital, with progressive improvement in renal function. The platelet count rose to 250 × 10⁹/l on the ninth day and the serum FDP concentration fell to normal on the 12th day. Four weeks after admission the blood pressure was normal and the ⁵¹Cr-EDTA clearance was 58 ml/min/1.73 m².

Comment

Activity of the prostacyclin was verified by testing the ability of aliquots of the solution to inhibit aggregation of normal platelets induced by adenosine diphosphate. The infusions were delivered via a central venous catheter, no antiplatelet agents were used, and no anti-hypertensive agents were required during treatment. Side effects were minimal and controllable. One patient developed intermittent drowsiness and vomiting; hypotension and bradycardia occurred only at a dose of more than 50 ng/kg/min and rapidly resolved after reduction in the rate of the infusion. The dose range (30-50 ng/kg/min) was appreciably greater than the maximum dose tolerated by adult patients with the haemolytic-uraemic syndrome (6 ng/kg/min)¹ and thrombotic thrombocytopenic purpura (16 ng/kg/min).²

Although these data are preliminary, they show the safety of this treatment in association with frequent blood-pressure monitoring. It is not possible to draw any conclusions on its success; the short oliguric phase in the last case, however, may have been related to the early introduction of the infusion at high dose. We feel sufficiently encouraged at this stage to continue with further evaluation of this treatment. Further experience is, however, essential, preferably on a controlled basis, before any recommendations on its use can be made.

Prostacyclin was synthesised by the Upjohn Company, formulated by the Wellcome Foundation, and kindly supplied by Dr J O'Grady.

¹ Webster J, Rees AJ, Lewis PJ, Hensby CN. Prostacyclin deficiency in haemolytic uraemic syndrome. *Br Med J* 1980;281:271.

² Hensby CN, Lewis PJ, Hilgard P, *et al.* Prostacyclin deficiency in thrombotic thrombocytopenic purpura. *Lancet* 1979;ii:748.

³ Budd GT, Bukowski RM, Lucas FV, *et al.* Prostacyclin therapy of thrombotic thrombocytopenic purpura. *Lancet* 1980;ii:915.

(Accepted 27 May 1981)

Renal Unit and Department of Haematology, Royal Hospital for Sick Children, Yorkhill, Glasgow G3 8SJ

T J BEATTIE, MB, MRCP, registrar in paediatrics (present appointment: senior registrar in paediatrics, Royal Manchester Children's Hospital, Pendelbury, Manchester M27 1HA)
A V MURPHY, MB, FRCP, consultant paediatric nephrologist
M L N WILLOUGHBY, MD, FRCPATH, consultant haematologist

Department of Medicine, Royal Infirmary, Glasgow G4 0SF

J J F BELCH, MB, MRCP, honorary registrar