Abnormal expression of glucose-6-phosphatase in preterm infants

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Abstract
The hepatic microsomal glucose-6-phosphatase enzyme was studied in liver samples from 76 premature infants including infants dying of sudden infant death syndrome. The data obtained were compared with glucose-6-phosphatase activity in liver samples from 95 term infants. In the majority of preterm infants up to 350 days of age the activity of the glucose-6-phosphatase enzyme was at or below the extreme low limit of the normal range in term infants. The premature infants with the lowest hepatic microsomal glucose-6-phosphatase activities are likely to be at risk of hypoglycaemic episodes during periods of relative starvation or stress.

(Arch Dis Child 1993;68:202-4)

It has been proposed that genetic deficiencies that have the potential to lead to severe hypoglycaemic episodes, if undiagnosed, may have the potential to cause sudden and unexpected death in infancy1 and many different biochemical abnormalities have been found in tissues removed at necropsy of infants dying of sudden infant death syndrome (SIDS).2-11 The hepatic microsomal glucose-6-phosphatase system catalyses the final step of the two pathways of liver glucose production (gluconeogenesis and glycogenolysis) and any deficiency resulting in abnormally low glucose-6-phosphatase activity has the potential to lead to hypoglycaemic episodes. We have previously reported 11 cases of abnormalities in the glucose-6-phosphatase system in association with raised hepatic glycogen in term infants who died suddenly and unexpectedly.12 Within this group there was one case of type 1a glycogen storage disease with no immunodetectable glucose-6-phosphatase protein; one case of type 1b and two cases of type 1c. Of the remaining seven cases what was surprising was that they all had abnormally low glucose-6-phosphatase enzyme activity and low amounts of immunodetectable glucose-6-phosphatase enzyme protein of normal molecular weight. A more recent study of the ontogeny of the glucose-6-phosphatase enzyme in term infants raises the possibility that some of those cases could be delayed or abnormal development of the glucose-6-phosphatase enzyme rather than cases of partial type 1a glycogen storage disease.13 Here we report that the pattern of abnormally low glucose-6-phosphatase enzyme activity with low amounts of immunodetectable glucose-6-phosphatase protein of normal molecular weight is common in infants born prematurely including infants succumbing to SIDS. Glucose-6-phosphatase activity is low in the first few postnatal days but persistence into infancy has not previously been described and could exacerbate the onset of hypoglycaemia in those infants at times of stress or starvation.

Patients and methods
PATIENTS
Liver tissue was obtained at necropsy from 116 premature infants (24-36 weeks’ gestation) who died up to 12 months after birth. Thirty two of the infants died within 24 hours of delivery and 20 died between 24 and 48 hours of delivery as a consequence of immature pulmonary function or intraventricular haemorrhage. Sixty four of the infants died later in the first year of life. In 32 of these cases no attributable cause of death could be found at necropsy and they have been considered to represent cases of SIDS. Control data from 95 term infants who were previously described have also been included.13 14 In early hospital deaths the mean interval between the time of death was pronounced and the time of necropsy was six hours (2-12 hours) during which time the bodies had been refrigerated. In some late postnatal deaths it was not possible to assess the specific time of death because some of the babies died suddenly and unexpectedly at home. In 171 out of the 211 liver samples described over 75% of the microsomes were intact on biochemical investigation and there was no evidence of even slight proteolysis.13-15 All liver samples that did not meet these two criteria were discarded (40 samples) leaving 76 samples from premature infants that were further analysed along with the 95 samples from term infants.

METHODS
Unfrozen hepatic samples were used to prepare microsomes as previously described.16 Glucose-6-phosphatase activity was assayed at 30°C as previously described.17 Microsomes were disrupted with histone 2A.18 Non-specific hydrolysis of glucose-6-phosphate was assayed and corrected for as in previous studies.17-19 The proportions of intact and disrupted microsomes were estimated by assays using 1 mmol/lmannose-6-phosphate as substrate for glucose-6-phosphatase and/or 1 naphthol as substrate for uridine diphasphate glucuronosyltransferase (both only produce activity in disrupted microsomes).20 21 Protein and glycogen were measured as described by Peterson22 and Van Handel23 respectively.
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Ethical approval for this study was given by the paediatric/reproductive medicine ethics of medicine research subcommittee of Lothian Health Board and by Tayside Health Board ethical committee.

Results
The activity of the glucose-6-phosphatase enzyme (measured in fully disrupted microsomes) in the majority of preterm infants between 0-5 days after birth remained low (below 0.2 μmol/min/mg protein) but a few infants in a 24 hour period around day 2 had higher levels of activity (fig 1). The pattern of glucose-6-phosphatase activity in preterm infants is quite different to the pattern of activity that we have previously shown in term infants. In term infants the activity of the glucose-6-phosphatase enzyme rises rapidly after birth and reaches normal adult activities (range 0.19-0.65 μmol/min/mg protein) within the first two to three days after birth. The majority of values of glucose-6-phosphatase activity in preterm infants between 0-5 days after birth (fig 1) are therefore lower than the values found in term infants of the same age. Hepatic glycogen content in the 96 term infants was less than 800 μg/mg protein in all samples. In the preterm infants, while the majority had a hepatic glycogen content less than 800 μg/mg protein some were not, perhaps, reflecting immaturity of hepatic glycogenolysis and gluconeogenesis. In term infants, from 3-352 postnatal days, the lower limit of the normal range of glucose-6-phosphatase activity was remarkably constant at approximately 0.2 μmol/min/mg protein (fig 2).

In contrast, over the same time period the vast majority of preterm infants were on, or below, the lower limit of the normal range (fig 2) with no obvious differences in glucose-6-phosphatase activity between cases with an attributable cause of death and those of SIDS.

Discussion
We have shown that abnormally low enzyme activity and expression of glucose-6-phosphatase enzyme in preterm infants is surprisingly common (figs 1 and 2). In contrast low activity is rare in full term infants, although this may be more common in those term infants who are categorised as having died of SIDS. In previous studies we have shown that in rat liver microsomes the glucose-6-phosphatase enzyme activity is very low at birth, but thereafter during the first few postnatal days, the activity of glucose-6-phosphatase enzyme overshoots to values several times higher than adult activities. In contrast, in term infants, the pattern is different with a rise from birth to adult values by 3 days of age, which are thereafter sustained—that is, there is no overshoot. Figure 1 shows that some preterm infants, around 2 days of age, have higher glucose-6-phosphatase activities than at any other time. This pattern is reminiscent of the postnatal overshoot in glucose-6-phosphatase activity previously seen in term rats and may reflect the relative precocity of term rats at birth compared with humans. How closely the postnatal development of glucose-6-phosphatase in preterm humans resembles that of term rats cannot be determined by serial liver biopsy for obvious ethical reasons.

In the majority of preterm infants after three postnatal days, the glucose-6-phosphatase enzyme activities are below the extreme limit of the normal term range with only a few infants above the lower extreme (fig 2). The high brain to body weight ratio in premature infants and the linear requirement of gestational brain size to glucose requirement would suggest that higher, not lower, hepatic glucose output is required in premature infants. Those preterm infants with low glucose-6-phosphatase values are likely to be at risk of hypoglycaemic episodes during periods of relative starvation or stress. This is critical for those preterm infants with glucose-6-phosphatase activities below 0.05 μmol/min/mg protein and within the range normally diagnostic for partial or complete type 1a glycogen storage disease.

We believe that the abnormal patterns of postnatal glucose-6-phosphatase enzyme activities in preterm infants are a likely consequence
of disordered perinatal regulation. It is not known how long this state of delayed or abnormal development persists, although we have previously reported a 19-year-old, who was born prematurely, and who had low glucose-6-phosphatase enzyme activity with low amounts of immunodetectable glucose-6-phosphatase protein. The risks of hypoglycaemia in infants are the same whether the deficiencies in the glucose-6-phosphatase system or indeed other key gluconeogenic enzymes are genetic or developmental in nature. We are now using animal models to model premature and term developmental delay with the aim of therapeutic manipulation of expression of glucose-6-phosphatase enzyme.

We thank Procurators Fiscal, Dr J M Anderson, Dr A D Bain, Dr J E Bell, Professor A Busuttil, Dr M W H Coughtrie, Dr M Giles, Mr A Glancy, Dr D Hopwood, Dr R T Jung, Dr L A Lang, Dr H Lyall, Dr J Peers, Dr A Shepherd, and Dr I D Waddell for tissue samples, help or advice, and Mrs L Gibb, Mrs P Houston, and Miss D Henderson for technical assistance.

This work was supported by a grant from the Scottish Home and Health Department to AB and RH and equipment grants from Tenovus (Scotland), Anonymous Trust, and Research Trust for Metabolic Diseases in Children to AB. AB is a Lister Institute Research fellow.

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