

# Inherited metabolic diseases in the sudden infant death syndrome

J B Holton, J T Allen, C A Green, S Partington, R E Gilbert, P J Berry

## Abstract

All sudden, unexpected infant deaths presenting during a two year period within a defined geographical area in Avon and north Somerset were investigated for inherited metabolic disease. Of 95 deaths, 88 were classified as cases of sudden infant death syndrome (SIDS). In addition to the normal postmortem investigations, samples of cerebrospinal fluid, urine, vitreous humour, and skin were collected for metabolic studies. No abnormal organic acid metabolites were found in the fluids from the 88 cases of SIDS. Fatty acid oxidation was assessed in skin fibroblasts from 70 cases of SIDS, but no examples of medium chain acyl CoA dehydrogenase (MCAD) deficiency were found. One case with abundant glycogen in the liver was subsequently diagnosed as having glycogen storage disease type 1c. These findings suggest that the incidence of MCAD deficiency and other metabolic diseases in SIDS is much lower than previously claimed.

A link between sudden, unexpected, infant deaths and inherited metabolic diseases was made almost 30 years ago,<sup>1</sup> but there has been increased interest in this topic recently.<sup>2</sup> In particular, it has been claimed that an abnormality of fatty acid  $\beta$ -oxidation, medium chain acyl CoA dehydrogenase (MCAD) deficiency, could cause 3% of cases of sudden infant death syndrome (SIDS),<sup>3</sup> and inherited metabolic diseases could account, in total, for about 10% of these deaths.<sup>4</sup> However, these estimates were not based on systematic studies of SIDS cases and may not represent the true contribution that metabolic diseases make to the overall problem.

Inherited metabolic diseases that are known to be associated with acute life threatening events are possible causes of SIDS.<sup>4</sup> The object of this study was to investigate all cases of sudden unexpected infant death within a defined geographical area over a two year period with a series of tests which should detect a large number of the relevant biochemical disorders.

## Methods

During a period of two years, starting in May 1987, there were 109 babies who died suddenly and unexpectedly within Avon and north Somerset. Of these, 14 were excluded from the study because of late notification, but their omission was not considered to bias the compo-

sition of the group. The median age of the remaining 95 cases was 94 days (range 7-551) with a male to female distribution of 1.6:1. As part of the postmortem examination performed on these cases, an initial set of specimens, comprising body fluids and microbiological cultures, were obtained within a median time of 3.5 hours (range 0.25-46) from discovery of death. These specimens were processed for microbiological investigations immediately, and the remainder of the sample was frozen at  $-70^{\circ}$  and stored for the biochemical tests. A skin biopsy specimen was taken for tissue culture and fibroblasts stored in liquid nitrogen at the second or third passage.

Table 1 reports the number of samples received from all 95 cases in the study. Urine was received from 54 (57%) of the patients, some of the samples obtained by squeezing out the nappy if the bladder was empty. Cerebrospinal fluid and vitreous humour was obtained in a higher proportion of babies. Skin samples were received from 80 cases and successful culture was achieved in 76 of them.

Further tissue samples were obtained at the usual postmortem examination, which followed the discovery of death by a median time of 25 hours (range 2.8-73). These tissues were investigated by a detailed histological protocol which included a frozen section of liver stained for neutral fat.<sup>5</sup> As a result of the basic postmortem findings the cause of death of seven cases in the study was considered to have been fully explained, while the remaining cases were classified as SIDS. However, the metabolic investigations were performed in all 95 cases in the study as far as adequate specimens were available.

Urine, cerebrospinal fluid, and vitreous humour were examined by gas chromatography/mass spectrometry (GC/MS) for organic acid metabolites<sup>6</sup> and by thin layer chromatography (TLC) for amino acids.<sup>7</sup> These are standard methods for detecting inherited metabolic diseases in the urine of living patients, GC/MS covering a wide range of fatty acid oxidation defects, organic acidurias, and some urea cycle disorders. It has been confirmed that a number of these diseases may be detected by the accumulation of abnormal metabolites in vitreous humour and cerebrospinal fluid obtained after death.<sup>8-10</sup> Similarly, TLC of these body fluids should detect all amino acidopathies and the urea cycle disorders that have increased amino acid concentrations. Available serum samples were analysed for free and total carnitine by a standard radioenzymatic method,<sup>11</sup> as a possible means of screening for organic acidurias.

Department of Clinical Chemistry,  
Southmead Hospital,  
Westbury-on-Trym,  
Bristol BS10 5NB  
J B Holton  
J T Allen  
C A Green  
S Partington

Bath Unit for Research into Paediatrics,  
Royal United Hospital,  
Bath  
R E Gilbert

Department of Paediatric Pathology,  
Royal Hospital for Sick Children,  
Bristol  
P J Berry

Correspondence to:  
Dr Holton.

Accepted 26 July 1991

Patients with organic acidurias tend to have low free carnitine concentrations because of an increased acylation of carnitine. A finding of low free carnitine, or an increased ratio of acyl to free carnitine, would indicate the need for more detailed investigation for an organic aciduria.

Cultured fibroblasts were used to measure MCAD by comparing  $^{14}\text{CO}_2$  release on incubation with (1- $^{14}\text{C}$ ) labelled octanoic acid with the release from labelled butyric acid by the method of Saudubray *et al.*,<sup>12</sup> as modified by G T N Butterworth and J Besley (personal communication).

### Results

The number of specimens in which the various analyses were performed are shown in table 1. Priority was given to GC/MS as it was considered to be the most likely to yield positive results. In all, 94 infants were investigated by GC/MS in at least one body fluid but no indication of a specific abnormality was found in any of the samples. There was a peak of acetylaspartic acid in 25 (59%) of the cerebrospinal fluid specimens. A similar abnormality has been reported in Canavan's disease,<sup>13</sup> a hereditary condition that is associated with spongy degeneration of the brain. There were no histological changes suggestive of this disease in our cases and it is almost certain that our finding was due to postmortem changes of no particular diagnostic importance.<sup>10</sup> There were no abnormalities in the TLC of amino acids.

Carnitine was determined in 52 serum specimens (table 2), of which 47 were in the SIDS category and five were explained. Concentra-

tions of free and total carnitine were grossly raised in most samples compared with reference values for live babies of similar age, whereas the ratios of acyl to free carnitine were comparable. There was no apparent difference between the explained deaths and the SIDS cases.

Table 3 gives the results of the skin fibroblast studies to diagnose MCAD deficiency. There was no difference between the results of 70 infants classified as SIDS and six in the explained death group. On the other hand, fibroblasts from four children not in the study, but diagnosed in our laboratory as having MCAD deficiency by GC/MS of urine and by other metabolic findings, showed a significant reduction in  $^{14}\text{CO}_2$  release with labelled octanoic acid as substrate. Table 3 shows a large standard deviation in all groups and there was an overlap in the distribution of the MCAD and the sudden death patients. However, this was due to a wide variation between batches in the assay, and within a batch patients with fatty oxidation defects were clearly differentiated.

Two livers have shown very striking panlobular microvesicular fatty changes comparable to those in Reye's syndrome, although no metabolic abnormality was found.

### Discussion

Our investigation of organic acids in urine, cerebrospinal fluid, and vitreous humour in 94 cases of sudden infant death, including 88 with SIDS, failed to detect any abnormality. In addition, there was no indication of MCAD deficiency in the 70 SIDS cases in which it was looked for specifically using skin fibroblasts. A deficiency of MCAD can be diagnosed usually both by the urinary organic acid profile and by the skin fibroblast methods. In all, 69 SIDS patients had both types of investigation and showed a concurrence of negative results. An almost identical study to our own, on 105 cases of SIDS, also produced negative results.<sup>10</sup> The number of patients we investigated by TLC of amino acids was rather limited, but a published study of quantitative amino acid concentrations on the vitreous humour of 120 SIDS patients failed to find any disorder.<sup>14</sup>

It has been suggested that the increase in serum carnitine observed in sudden infant death (table 2) is a postmortem change, because a correlation between carnitine concentration and the interval from estimated time of death to blood sampling has been found (P Divry, personal communication). Our data showed a similar tentative correlation. The estimated time of death was calculated as the mid-point in the time interval from when the baby was last known to be alive to when it was found dead. Babies were included in the analysis when this time interval was less than six hours ( $n=36$ ). The correlation between the plasma free carnitine and the time from the estimated death to the blood sample being taken gave a linear regression equation,  $y=36.1+24.4x$  ( $r=0.61$ ,  $p<0.001$ ). For plasma total carnitine the regression equation was  $y=91.4+25.7x$  ( $r=0.67$ ,  $p<0.001$ ). It seems probable that the post-mortem rise in serum carnitine would mask a

Table 1 Samples received and analysed from 95 cases of sudden infant death

	Cerebrospinal fluid	Urine	Vitreous humour	Blood	Skin
Samples received	77	54	84	55	80
Samples analysed					
Organic acids (by GC/MS)	42	47	84	—	—
Amino acids (by TLC)	12	5	0	—	—
Carnitine in serum	—	—	—	52	—
$\text{CO}_2$ release from octanoate in skin fibroblasts	—	—	—	—	76

A blank indicates that the assay was not applicable in the type of sample.

Table 2 Free and total serum carnitine concentrations in 52 cases of sudden infant death. Results are mean (range)

	No of babies	Free carnitine ( $\mu\text{mol/l}$ )	Total carnitine ( $\mu\text{mol/l}$ )	Ratio acyl*/free carnitine
Sudden infant death	52	169 (66-550)	235 (79-620)	0.42 (0.12-0.76)
Reference values <sup>1</sup> (babies aged 8 days-1 year)	25	33 (15-51)	44 (19-68)	0.36 (0.11-0.65)

\*Acyl=total-free carnitine.

Table 3 Medium chain acyl CoA dehydrogenase activity in skin fibroblasts (nmol/h/mg protein). Results are mean (SD)

	No of babies	$^{14}\text{CO}_2$ release from octanoate	$^{14}\text{CO}_2$ release from butyrate	Ratio octanoate/butyrate oxidation
SIDS	70	1.43 (0.57)	2.84 (0.85)	0.50 (0.14)
Explained sudden death	6	1.15 (0.60)	2.56 (1.02)	0.46 (0.18)
Known MCAD	4	0.47 (0.14)	2.76 (0.79)	0.17 (0.05)

There is a significant difference between the SIDS and MCAD deficiency cases for  $^{14}\text{CO}_2$  release from octanoate ( $p<0.01$ ) and for the ratio of octanoate to butyrate oxidation ( $p<0.001$ ).

free carnitine deficiency that should indicate the presence of an organic aciduria. However, table 2 shows that the ratio of acyl to free carnitine remained normal in our sudden death patients and therefore it is possible that a high ratio could successfully indicate an organic aciduria. This hypothesis could only be tested when positive cases of organic aciduria are found in SIDS.

The results reported here and by others<sup>10-14</sup> suggest that the incidence of MCAD deficiency and other metabolic diseases in SIDS is very much lower than previously estimated. Despite this, it is important to identify the rare cases in which inherited metabolic disease is the cause of death. Routine screening of all sudden infant deaths may not be justifiable and some means of concentrating investigations of those babies with a higher likelihood of having an underlying metabolic disease would be valuable. The acute episode in a metabolic disease is almost always preceded by a short illness with febrile, particularly gastrointestinal, features which affects regular food intake. Unfortunately, similar signs of illness are described in the week before death in many SIDS cases, notably in two thirds of the babies included in our study.<sup>15</sup> The finding of panlobular microvesicular fatty changes in the liver at postmortem examination is another factor which needs to be considered. It may suggest the presence of a metabolic disorder before death, but it is not a specific indication of this<sup>16</sup>; nor it is clear that the absence of liver fatty changes rule out the possibility of a metabolic disease.<sup>17</sup> One has to conclude that there are no satisfactory indicators of inherited metabolic disease in SIDS cases apart from the rare circumstances of the unexplained death of a sibling.

Since the completion of this study, interest was aroused by a report linking glucose-6-phosphatase deficiency (glycogen storage disease type 1, GSD 1) with sudden infant deaths.<sup>18</sup> One of the patients in our study was noted to have an unusually high lactic aciduria and abundant glycogen in the liver. This case has now been diagnosed as GSD 1c by A Burchell

(personal communication). Liver from all cases in our study has now been investigated for GSD 1, with a negative result.

We wish to thank Drs PT Rudd, PJ Fleming, and E Hall for their important contributions to this study and the Foundation for the Study of Infant Deaths for a grant supporting CAG and REG.

- 1 Cleveland WW, Green OC, Wilkins L. Deaths in congenital adrenal hyperplasia. *Pediatrics* 1962;29:3-17.
- 2 Anonymous. Sudden infant death and inherited disorders of fat oxidation. *Lancet* 1986;ii:1073-5.
- 3 Howat AJ, Bennett MJ, Variend S, Shaw L. Deficiency of medium chain acylcoenzyme A dehydrogenase presenting as sudden infant death syndrome. *BMJ* 1984;288:976.
- 4 Emery JE, Howat AJ, Variend S, Vawter GF. Investigation of inborn errors of metabolism in unexpected infant deaths. *Lancet* 1988;ii:29-31.
- 5 Wrigglesworth JS, Keeling JW, Rushton DI, Berry PJ. Pathological investigations in cases of sudden infant death syndrome. *J Clin Pathol* 1987;40:1481-3.
- 6 Chalmers RA, Lawson AM. *Organic acids in man*. London: Chapman and Hall, 1982:11-135.
- 7 Ersser RS, Smith I. Amino acids and related compounds. In: Smith I, Seakins JWT, eds. *Chromatographic and electro-phoretic techniques*. Vol 1. London: Heinemann, 1976: 75-108.
- 8 Bennett MJ, Marlow N, Politt RJ, Wales JKH. Glutaric aciduria type 1: biochemical investigations and post-mortem findings. *Eur J Pediatr* 1986;145:403-5.
- 9 Coude M, Bonnefont JP, Charpentier C, Chadefaux B, Saudubray JM, Kamoun P. Aqueous humour, as possible material for post-mortem methylmalonic aciduria diagnosis. *J Inherited Metab Dis* 1988;12:95-6.
- 10 Divry P, Vianey-Liaud C, Jakobs C, Ten-Brink HJ, Dutruge J, Gilly R. Sudden infant death syndrome: organic acid profiles in cerebrospinal fluid from 47 children and the occurrence of N-acetylaspartic acid. *J Inherited Metab Dis* 1990;13:330-2.
- 11 Schmidt-Sommerfeld E, Werner D, Penn D. Carnitine plasma concentrations in 353 metabolically healthy children. *Eur J Pediatr* 1988;147:356-60.
- 12 Saudubray J-M, Coude F-X, Demangre F, Johnson C, Gibson KM, Nyhan WL. Oxidation of fatty acids in cultured fibroblasts: a model system for the detection and study of defects in oxidation. *Pediatr Res* 1982;16:877-81.
- 13 Matalon R, Michals K, Sebesta D, Deanching M, Gashkoff P, Casanove J. Aspartoacylase deficiency and N-acetyl-aspartic aciduria in patients with Canavan disease. *Am J Med Genet* 1988;29:463-71.
- 14 Patrick WJA, Logan RW. Free amino acid content of vitreous humour in cot deaths. *Arch Dis Child* 1988;63: 660-3.
- 15 Gilbert RE, Fleming PJ, Azaz Y, Rudd PT. Signs of illness in babies preceding sudden unexplained infant death. *BMJ* 1990;300:1237-9.
- 16 Bonnell HJ, Beckwith JB. Fatty liver in sudden childhood death: implications for Reye's syndrome? *Am J Dis Child* 1986;140:30-3.
- 17 Losty HC, Lee P, Alfaham M, Gray OP, Leonard JV. Fatty infiltration in the liver of medium chain acyl CoA dehydrogenase deficiency. *Arch Dis Child* 1991;66:727-8.
- 18 Burchell A, Bell JE, Busuttill A, Hume R. Hepatic microsomal glucose-6-phosphatase system and sudden infant death syndrome. *Lancet* 1989;ii:291-4.