

## Leading articles

# Reye syndrome—insights on causation and prognosis

Reye syndrome (RS) is an abrupt insult to mitochondria manifesting as acute encephalopathy, selective hepatic dysfunction, and fatty infiltration of the viscera—as originally described in 1963 (see Wood<sup>1</sup>). Causation, however, remains unclear, especially the role of aspirin in possible pathogenesis.<sup>1</sup> Although prompt recognition and intensive therapy is essential to full recovery, a paucity of cases in recent years has meant that few younger paediatricians have any personal experience. This and a spate of recent literature—clinical and scientific—has prompted this review.

### Background

RS is a biphasic illness. A viral prodrome—with infection of the upper respiratory tract or bowel, or varicella—is followed several days later by an abrupt onset of encephalopathy heralded as profuse, effortless vomiting. The rate and degree of neurological decline vary. Raised intracranial pressure from brain swelling is usually thought to cause death or neurological injury.

The British Paediatric Surveillance Unit defines RS as an unexplained, non-inflammatory encephalopathy in those less than 16 year of age, associated with a serum aspartate or alanine aminotransferases, or plasma ammonia more than three times the normal limit, or hepatic fatty infiltration that is microvesicular in appearance and panlobular in distribution.<sup>2</sup> Diagnostic criteria are non-specific. One group maintains that antiemetics taken after onset of the vomiting have a causal role in RS, another disputes its actual existence<sup>3 4</sup>; evidence in support of these views is unconvincing and they have gained little support. However, a proportion of those thought initially to have RS (12%) are shown later to have an inherited metabolic disorder (IMD), such as single enzyme defects of  $\beta$  oxidation, or the urea cycle.<sup>2</sup> “Classical” RS must of necessity be a diagnosis of exclusion.

### Aspirin—a co-factor?

The evidence on prodromal aspirin and RS has been reviewed.<sup>5</sup> Several case-control studies assert that the association is strong, consistent, and unbiased; this is especially true of the very rigorous “Yale” study, in which a dose response effect was also noted.<sup>6</sup> Even a *total* dose less than 45 mg/kg increased the risk of RS by 20-fold—the authors concluding that “no safe dose [of aspirin] exists”. No other exogenous agent has been implicated in similar well designed studies (see Hall<sup>5</sup>).

Longitudinal surveillance provides further evidence. A recent review of 1207 cases reported to the National RS Surveillance System (NRSSS) at the Centers for Disease Control and Prevention, Atlanta, USA (1 December 1980 to 30 November 1997) identified 555 cases in 1980, but only two annually between 1994 and 1997<sup>7</sup>; case reporting was to the 18th birthday. In the UK, the annual incidence declined from 81 cases in 1983–84 to five in 1996–97.<sup>2</sup> One elegant study showed that this decline mirrored the upsurge in publications mentioning the link with aspirin that began in the USA in 1980.<sup>8</sup> Furthermore, in Northern Ireland, febrile children admitted to hospital in 1988–89 showed a 17-fold reduction in preadmission aspirin use

compared to those in 1985–86 (the Committee on Safety of Medicines warned of the aspirin risk in mid-1986).

Finally, more than 400 UK cases reported between 1982 and 1990 were reviewed “blindly” using a Reye score (devised by Dr Susan Hall to quantitate features characteristic of “classical” RS) employed here in modified form. Highest scores were achieved by a distinct aspirin related subgroup that diminished significantly in number (79%) after 1986.<sup>9</sup> The evidence of an association therefore seems epidemiologically sound, and the disappearance of RS consequent on the aspirin warnings was described two years ago in an editorial in the *New England Journal of Medicine* as “a public health triumph”.<sup>10</sup>

### Aspirin metabolism

Following absorption, aspirin is metabolised rapidly to salicylate. At usual anti-inflammatory doses, with plasma concentrations of 1–5 mM, the half life approximates to 12 hours; at high therapeutic doses it may reach 30 hours. Salicylate is metabolised within hepatocyte mitochondria and endoplasmic reticulum to significant amounts of hydroxyhippurate (HHA) and gentisate. Among 56 patients with RS in Northern Ireland (1979–86), the serum salicylate range and mean on admission were 0–2.5 and 0.9 mM/l, respectively (JG, unpublished data). This concurs with published data<sup>1</sup>; HHA concentrations tend to be twofold higher.<sup>11</sup> In RS, therefore, aspirin metabolites may persist for some time at millimolar concentrations. Are such concentrations capable of influencing events, however?

### Viral infection and the immune response

Antecedent viral infection appears to be a *sine qua non* for development of RS.<sup>1</sup> Viral RNA frequently diverts host endoplasmic reticulum to biosynthesis of viral protein.<sup>1</sup> A number of virus infections disturb Kupffer cell function and endotoxaemia commonly results, triggering a cascade of cytokines.<sup>12</sup> Endotoxin like activity has been reported in plasma and CSF in RS.<sup>13</sup> Endotoxin provokes release by macrophages of tumour necrosis factor (TNF), a known mediator of metabolic toxicity. In rat hepatocytes, for example, TNF inhibits fatty acid oxidation; influenza B virus produces similar effects in mice. Following sublethal doses of endotoxin, rats have increased concentrations of plasma ammonia and free fatty acids, and develop hepatic fatty infiltration and mitochondrial damage. Immature rodents appear more sensitive to the effects of TNF.<sup>14</sup> Aspirin enhances *in vitro* release of TNF by mouse macrophages.<sup>14</sup> Finally, aspirin and salicylate at 1–5 mM directly inhibit release of the proinflammatory and antiapoptotic nuclear factor  $\kappa$ B, allowing target cells to proceed more rapidly down the pathway to cell death.<sup>15</sup> Thus viral infection may influence cellular and subcellular events via immune mediators.

### Mitochondrial failure

The clinical findings in RS are compatible with hepatotoxicity caused by mitochondrial failure.<sup>16</sup> Mitochondria provide most of the energy for liver cell function via oxidative phosphorylation fed by the tricarboxylic acid cycle and

$\beta$  oxidation of long chain fatty acids (LCFA). Mitochondria are also closely involved in cell death processes such as the mitochondrial permeability transition (MPT). This is the opening of a cyclosporin sensitive pore in the inner membrane of mitochondria leading to swelling, depolarisation, failure of oxidative phosphorylation, and cell death by apoptosis or necrosis.<sup>17</sup> Recently, Lemasters' group has shown that 0.3 mM salicylate can initiate MPT in isolated liver mitochondria,<sup>18</sup> and, in cultured rat hepatocytes, 0.3–5 mM salicylate enhanced cell killing.<sup>19</sup>

In RS the burden of evidence suggests that mitochondrial failure is the result of inhibition of oxidative phosphorylation and fatty acid  $\beta$  oxidation of LCFA.<sup>1</sup> Microvesicular steatosis is a likely consequence and accumulation of tissue LCFA has recently been shown to initiate apoptosis via increased ceramide in a variety of cell types.<sup>20, 21</sup> Another factor is that toxic acyl-CoA esters accumulate within mitochondria and sequester free coenzyme A; the development of hyperammonaemia and hypoglycaemia are but two examples that illustrate the presence of generalised mitochondrial dysfunction.<sup>1</sup> Thus inhibition of mitochondrial  $\beta$  oxidation in RS could lead to severe liver dysfunction and fatty infiltration.

### Salicylate blocks $\beta$ oxidation of long chain fatty acids

In the first study to use human cells, aspirin metabolites (though not aspirin itself) have been shown recently to inhibit mitochondrial  $\beta$  oxidation of palmitate.<sup>22</sup> This was carried out in skin fibroblasts from children who had recovered from RS (also given aspirin for prodromal symptoms), and from controls. In cells from the latter, two of the principal metabolites—hydroxyhippurate (HHA) and gentisate—were more inhibitory than salicylate at concentrations below 5 mM, but RS cells were equally sensitive to all aspirin metabolites. Thus salicylate below 5 mM was a more effective inhibitor of  $\beta$  oxidation in RS cells than in controls.

### Where is the site of inhibition?

Structural similarities between aspirin metabolites and substrates for the mitochondrial trifunctional enzyme (MTE) of  $\beta$  oxidation prompted us to examine whether the long chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) component of MTE was the target for salicylate inhibition. Using fibroblasts from individuals with LCHAD deficiency, it was possible to show complete absence of inhibition of  $\beta$  oxidation by salicylate or HHA compared to control cells or RS cells, thus indicating that the LCHAD enzyme is the target for such inhibition.<sup>22</sup>

### A biological difference between control and Reye syndrome cells?

Our studies have also shown that fibroblasts from RS patients were significantly more sensitive to inhibition of  $\beta$  oxidation by salicylate than are control cells. The differences were demonstrable at concentrations well within the therapeutic range of plasma salicylate (see above). In control cells, 1 mM salicylate significantly *stimulated* palmitate oxidation, an effect quite opposite to that noted in RS cells, higher concentrations caused increasing inhibition in each group of cells.<sup>22</sup> Stimulatory then inhibitory effects of salicylate on  $\beta$  oxidation were reported at similar concentrations in rat liver slices.<sup>23</sup> Thus stimulatory and inhibitory effects of salicylate were independent; only the latter was due to blocking of LCHAD activity.

How might this stimulation of  $\beta$  oxidation, at therapeutic salicylate concentrations, be induced? Salicylate, though not HHA or gentisate, is known to uncouple mitochondrial  $\beta$  oxidation from phosphorylation. Thus the stimulatory

effects in control cells might be due to the presence of an uncoupling protein. In RS cells, it is possible that salicylate does not uncouple oxidative phosphorylation because a specific target protein is absent. This target protein we suggest is probably one of the family of mitochondrial uncoupler proteins (UCPs)<sup>24</sup> whose role is still unclear. Their expression is increased under conditions in which LCFA accumulate, prompting the suggestion that, by stimulating mitochondrial respiration<sup>25</sup> they enhance  $\beta$  oxidation, thus protecting against apoptosis (see above). Lack of a specific UCP, however caused, might explain the difference in  $\beta$  oxidation response to low concentrations of salicylate between controls and LCHAD deficient cells on the one hand, and RS cells on the other—a difference that warrants further investigation. This sensitivity of RS cells, if widely expressed in body tissues, could explain the reaction of certain individuals to the action of aspirin on the MTE-LCHAD enzyme system that might contribute to the pathogenesis of RS.

### Prognosis

If the neurological phase of RS resolves, visceral function is expected to recover completely within a few days. However, the severity and duration of both metabolic and hydrostatic effects on the nervous system will determine prognosis.<sup>1</sup> Although pathogenesis of the brain swelling is unclear, its results can be all too evident, with elevation of intracranial pressure, and, if more severe, reduction in cerebral perfusion pressure; a cerebral perfusion pressure of 40 mm Hg or less in RS carries a very poor outcome. In the NRSSS, even small biochemical abnormalities appeared to influence mortality. Logistic regression analysis showed that, surprisingly, a blood ammonia little greater than the normal upper limit for older children was significantly associated with a risk of demise ( $p < 0.01$ )—the relative risk of death being 3.4 and of neurological complications 4.1.<sup>7</sup>

In our series, more than 70% of the children achieved a good initial outcome (62% in NRSSS<sup>7</sup>). This optimism must be interpreted with caution because in the UK, RS has tended to occur at a very young age (median age 14 months<sup>9</sup>; median age in NRSSS, 6 years<sup>7</sup>). Damage to the developing brain that results in neuropsychological deficits may only become apparent much later when maturation deviates from normal, and abilities may be simply less well acquired. Moreover mild impairments, perhaps clinically undetectable in themselves, may exert a cumulative influence on the development of other functions, leading to more significant disabilities.<sup>26</sup> Hence the need for outcome studies during the school years and beyond. Among 56 patients seen between 1979 and 1986 (median age 0.8 years), Meekin and colleagues studied cognitive, emotional, and behavioural sequelae in 18 children who had recovered well enough to attend normal schools. The mean age was then 11.75 years, with each child being compared to the sibling nearest in age.<sup>27</sup> On the basis of formal testing, general intellectual functioning, and verbal, and visuospatial skills were significantly reduced if RS occurred during infancy. A similar profile was evident on the measure of self esteem. Differences for measures of behavioural problems failed to reach significance. Another significant variable was the development of unconsciousness during encephalopathy. Although this was not so closely associated with less good outcomes, it was linked to relative deficits on the scale of cognitive ability; however, only those differences on the Wechsler scale of verbal abilities reached statistical significance.

## Conclusion

Long term follow up in RS may in its way be as important as early recognition and optimum management, as are diagnostic investigations aimed at uncovering an IMD. Inhibition of the MTE-LCHAD enzyme system by salicylate and metabolites, and the differences shown for the first time in human material between control and RS cells, lends biological plausibility to the substantial body of epidemiological evidence linking RS with aspirin use during childhood viral illness. Our view is that current warnings about aspirin use must be maintained—with continued case monitoring—to ensure that this potentially devastating encephalopathy does not reappear and that “the public health triumph” will also be fully realised in the UK.

We wish to record thanks to the National Reye's Syndrome Foundation of the UK for generous research support.

J F T GLASGOW

Department of Child Health, The Queen's University of Belfast and Royal Belfast Hospital for Sick Children

B MIDDLETON

School of Biomedical Sciences, The Medical School, University of Nottingham

Correspondence to: Dr J F T Glasgow, Royal Belfast Hospital for Sick Children, Falls Road, Belfast BT12 6BE, Northern Ireland  
john.glasgow@royalhospitals.n-i.nhs.uk

- 1 Wood C, ed. *Reye's syndrome*. Round Table Series 8. London: Royal Society of Medicine Services, 1988:1,80–3,16,45,64,25,135,153–4.
- 2 Hall SM, Lynn R. Reye syndrome. In: Guy M, Nicoll A, Lynn R, eds. *British Paediatric Surveillance Unit, 12th Annual Report*. London: RCPCH, 1998.
- 3 Casteels-Van Daele M. Reye syndrome or side-effects of anti-emetics? *Eur J Pediatr* 1991;150:456–9.
- 4 Orłowski JP. Whatever happened to Reye syndrome? Did it ever really exist? *Crit Care Med* 1999;27:1582–7.
- 5 Hall SM. Reye's syndrome and aspirin: a review. *Br J Clin Pract* 1990;44(suppl 70):4–11.
- 6 Forsyth BW, Horwitz RI, Acampora D, et al. New epidemiologic evidence confirming that bias does not explain the aspirin/Reye's syndrome association. *JAMA* 1989;261:2517–24.
- 7 Belay ED, Bresee JS, Holman RC, et al. Reye's syndrome in the United States from 1981 through 1997. *N Engl J Med* 1999;340:1377–82.
- 8 Soumerai SB, Ross-Degnan D, Kahn JS. Effects of professional and media warnings about the association between aspirin use in children and Reye's syndrome. *Milbank Quarterly* 1992;70:155–82.
- 9 Hardie RM, Newton LH, Bruce JC, et al. The changing clinical pattern of Reye's syndrome 1982–1990. *Arch Dis Child* 1996;74:400–5.
- 10 Monto AS. The disappearance of Reye's syndrome—a public health triumph. *N Engl J Med* 1999;340:1423–4.
- 11 Meert KL, Kauffman RE, Deshmukh DR, Sarnaik AP. Impaired oxidative metabolism of salicylate in Reye's syndrome. *Dev Pharmacol Ther* 1990;15:57–60.
- 12 Herbein G, O'Brien WA. Tumor necrosis factor-alpha and TNF receptors. *Proc Soc Exp Biol Med* 2000;223:241–57.
- 13 Cooperstock MS, Tucker RP, Baublis JV. Possible pathogenetic role of endotoxin in Reye's syndrome. *Lancet* 1975;1:1272–4.
- 14 Larrick JW, Kunkel SL. Is Reye's syndrome caused by augmented release of tumour necrosis factor? *Lancet* 1986;2:132–3.
- 15 Yin M-J, Yamamoto Y, Gaynor RB. The anti-inflammatory agents aspirin and salicylate inhibit activity of IκB kinase-β. *Nature* 1998;396:77–80.
- 16 Pessayre D, Mansouri A, Haouzi D, Fromenty B. Hepatotoxicity due to mitochondrial dysfunction. *Cell Biol Toxicol* 1999;15:367–73.
- 17 Halestrap AP. The mitochondrial permeability transition: its molecular mechanism and role in reperfusion injury. In: Brown GC, Nicholls DG, Cooper CE, eds. *Mitochondria and cell death*. London: Portland Press, 1999:181–203.
- 18 Trost LC, Lemasters JJ. The mitochondrial permeability transition: a new pathophysiological mechanism for Reye's syndrome and toxic liver injury. *J Pharmacol Exp Ther* 1996;278:1000–5.
- 19 Trost LC, Lemasters JJ. Role of the mitochondrial permeability transition in salicylate toxicity to cultured rat hepatocytes: implications for the pathogenesis of Reye's syndrome. *Toxicol Appl Pharmacol* 1997;147:431–41.
- 20 Paumen M, Ishida Y, Muramatsu M, et al. Inhibition of carnitine palmitoyl-transferase I augments sphingolipid synthesis and palmitate-induced apoptosis. *J Biol Chem* 1997;272:3324–9.
- 21 Chan TA, Morin PJ, Vogelstein B, Kinzler KW. Mechanisms underlying nonsteroidal antiinflammatory drug-mediated apoptosis. *Proc Natl Acad Sci USA* 1998;95:681–6.
- 22 Glasgow JFT, Middleton B, Moore R, et al. The mechanism of inhibition of β-oxidation by aspirin metabolites in skin fibroblasts from Reye's syndrome patients and controls. *Biochem Biophys Acta* 1999;1454:115–25.
- 23 Maddaiah VT, Miller PS. Effects of ammonium chloride, salicylate, and carnitine on palmitic acid oxidation in rat liver slices. *Pediatr Res* 1989;25:119–23.
- 24 Ricquier D, Bouillaud F. The uncoupling protein homologues: UCP1, UCP2, UCP3, StUCP and AtUCP. *Biochem J* 2000;345:161–79.
- 25 Boss O, Hagen T, Lowell BB. Uncoupling proteins 2 and 3: potential regulators of mitochondrial energy metabolism. *Diabetes* 2000;49:143–56.
- 26 Baron IS, Fennell EB, Voeller K. *Pediatric neuropsychology in the medical setting*. New York: Oxford University Press, 1995.
- 27 Meekin S, McCusker C, Glasgow JFT, Rooney N. A long-term follow-up of cognitive, emotional and behavioural sequelae to Reye's syndrome. *Dev Med Child Neurol* 1999;41:549–53.

## ADC online submission and review system

The Editors of *ADC* are pleased to inform authors and reviewers of its new online submission and review system. Developed by Highwire Press (CA, USA), *Bench>Press* is a fully integrated electronic system which uses the internet to allow rapid and efficient submission of manuscripts, and for the peer review process to be conducted entirely online. We are the first journal of the BMJ publishing group to go online in this way; the aim, apart from saving trees, is to speed up the frequently frustrating progress from submission to publication.

Authors can submit their manuscript in any standard word processing software. Standard graphic formats acceptable include: .jpg, .tiff, .gif, eps, etc. The text and graphic files are automatically converted to PDF for ease of distribution and reviewing purposes. Authors are asked to approve their submission before it formally enters the reviewing process. On approval, the submission is passed to the editor and/or reviewers via the web. All transactions are secure.

To access the system click on “SUBMIT YOUR MANUSCRIPT HERE” on the ADC homepage: <http://www.archdischild.com>, or you can access the submission site directly at <http://submit-adc.bmjournals.com>.

We are very excited with this new development and would encourage authors and reviewers to use the system where possible. It is simple to use and should greatly improve on the current peer review process. Full instructions can be found on *Bench>Press* and *ADC online*.



## Reye syndrome—insights on causation and prognosis

J F T GLASGOW and B MIDDLETON

*Arch Dis Child* 2001 85: 351-353  
doi: 10.1136/adc.85.5.351

---

Updated information and services can be found at:  
<http://adc.bmj.com/content/85/5/351>

---

	<i>These include:</i>
<b>References</b>	This article cites 23 articles, 6 of which you can access for free at: <a href="http://adc.bmj.com/content/85/5/351#BIBL">http://adc.bmj.com/content/85/5/351#BIBL</a>
<b>Email alerting service</b>	Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

---

### Topic Collections

Articles on similar topics can be found in the following collections

[Liver disease](#) (161)  
[Metabolic disorders](#) (673)  
[Epidemiologic studies](#) (1577)

---

### Notes

---

To request permissions go to:  
<http://group.bmj.com/group/rights-licensing/permissions>

To order reprints go to:  
<http://journals.bmj.com/cgi/reprintform>

To subscribe to BMJ go to:  
<http://group.bmj.com/subscribe/>