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Reye's Syndrome
The Case For a Causal Link with Aspirin

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Abstract

Reye's syndrome is a serious, acute encephalopathy that has been linked with aspirin (acetylsalicylic acid) use in children and teenagers <18 years of age. Although others may disagree, it is my belief that any objective analysis of published material in the last 20 years must conclude that there is a close link between the devastating encephalopathy Reye's syndrome and ingestion of aspirin during the febrile prodrome.

The drug appears to act as a co-factor in susceptible individuals. Although some of the epidemiological data indicate an association between the two, the burden of evidence suggests actual causality and is both consistent and specific as well as strong and time related. Some of the evidence points to illness severity being dose related although it seems that in the presence of a viral infection, no dose of aspirin can be considered safe. No published work, using methodology that can be critically evaluated, has shown evidence to contradict these conclusions and they have been widely accepted. Since government health warnings were appended to aspirin-containing formulations, the decline in case numbers on both sides of the Atlantic has been nothing short of remarkable. Recent *in vitro* findings have pinpointed the site of action of the drug on the long chain hydroxyacyl-CoA dehydrogenase enzyme (a component of the mitochondrial trifunctional enzyme) and, even at therapeutic concentrations, oxidation is impaired in cultured fibroblasts from patients who have recovered from the disorder. This is quite unlike that seen in cells from normal controls.

Even when major influenza outbreaks occur in the future, Reye's syndrome is preventable provided government health warnings are heeded and the cogent evidence set forth here is acted upon by the parents of feverish children and self-medicating teenagers.

Reye's syndrome is a serious, acute encephalopathy associated with selective hepatic dysfunction that can affect children, teenagers and sometimes adults. The primary insult is to mitochondria and there are ultrastructural, histochemical and pathophysiological abnormalities to support this.

The clinical presentation of Reye's syndrome begins like any other intercurrent viral infection with flu-like, upper respiratory or gastrointestinal symptoms; in the US the syndrome has been linked more specifically with influenza (especially flu B) or varicella.[1] A few days later an abrupt deterioration occurs, with repeated, profuse vomiting (often of altered blood) that appears to mark the onset of

Table I. Published US case-control studies showing an association between Reye's syndrome and aspirin and a lack of association with paracetamol (acetaminophen) use

Study	Study date	No. of cases	No. of controls	% salicylate use (cases : controls)	OR	% paracetamol use (cases : controls)	OR
Starko et al.[5]	1978	7	16	100 : 50			
Waldman et al.[6]	1980	25	46	97 : 57			
Waldman et al.[6]	1980–1	12	29				
Halpin et al.[7]	1978–80	97	156	97 : 71	11	16 : 33	
Hurwitz et al.,[8] pilot PHS study	1984	30	145	93 : 46	16	27 : 67	
Hurwitz et al.,[9] main PHS study	1985–6	27	140	96 : 38	40	30 : 86	0.06
Forsyth et al.,[10] the Yale study	1986–7	24	48	88 : 17	35	38 : 71	0.16

OR = odds ratio; **PHS** = Public Health Service.

encephalopathy. Patients also experience varying degrees of reduced consciousness.

The purpose of this article is to use epidemiological[2] and experimental[3] evidence to make the case for a strong link between Reye's syndrome and aspirin (acetylsalicylic acid) usage during the febrile prodrome and, in so doing, to rebut suggestions that the evidence linking the two is questionable.[4] My argument is set within historical and political contexts of the times, the actions taken by various governments and the effect of these decisions. Moreover, this topic is particularly relevant at present, as an influenza outbreak or pandemic seems possible because of the emergence of the highly pathogenic A/H5/N1 virus that has infected poultry in East Asia since 2003.

1. Reye's Syndrome and Aspirin

Although several factors are needed for the development of Reye's syndrome in susceptible individuals, aspirin is now widely recognised as an essential co-factor that, together with a prodromal viral infection/cytokine response, results in a massive catabolic reaction. The role of aspirin is derived from epidemiological data and, much more recently, from *in vitro* research. Of the former, two types of study (descriptive and analytical) have elucidated the principal risks due to aspirin. The latter have consisted of case-control studies in which patients with Reye's syndrome were compared with symptomatic controls who did not develop the syndrome.

The initial studies linking Reye's syndrome and aspirin therapy were the series of case-control investigations conducted in the US between 1978 and 1987.[5-9] Two years later, these were followed by the paper of Forsyth et al.,[10] which will be referred to in more detail in section 2. In patients with Reye's syndrome, all six studies demonstrated a significant excess risk of salicylate usage (specified as aspirin in four studies) during the prodromal illness (table I) and provided the initial evidence of the dangers of aspirin therapy for febrile illnesses.

In 20 years surveillance in the UK and Ireland, the work of Dr Susan Hall has been crucial in organising two consecutive, prospective surveys (table II). The British Reye's Syndrome Surveillance Scheme (BRSSS) was followed in July 1986 by an 'active' reporting system, based at the British Paediatric Surveillance Unit (BPSU) of the British Paediatric Association – now the Royal College of Paediatrics and Child Health. It also utilised data from the Office for National Statistics and its equivalents for Scotland and Northern Ireland.

Since the definitive publication by Dr Reye et al.[11] was published in 1963, my colleagues and I have seen approximately 80 patients with Reye's syndrome in Northern Ireland, 56 of whom were reviewed for a national workshop entitled Reye's Syndrome and Reye-like Inherited Metabolic Disorders in 2002;[12] 23 of these were reported previously.[13] In the UK and Ireland, 632 cases were reported to a national reporting scheme (1981–2001), of which 450 cases were confirmed (table II).[14] There were 239 (53%) deaths.

Diagnostic criteria used for the surveillance studies in the UK and Ireland (collecting data from patients aged <16 years) are very non-specific and consist of an unexplained, noninflammatory encephalopathy that is associated with a serum aspartate or alanine aminotransferase or plasma ammonia level more than three times the normal limit, or hepatic fatty infiltration that is microvesicular in microscopic appearance and pan lobular in distribution.[2] A proportion of those patients thought initially to have Reye's syndrome (12%) were later shown to have an inherited metabolic disorder, such as single-enzyme defects of β-oxidation or the urea cycle (table II).[14] Because Reye's syndrome is a diagnosis of exclusion, immediate investigations to this end are considered crucial.

2. Resulting Reaction in US and Political Action

The findings of the first three US studies[5-7] led the US Surgeon General to issue a public and professional warning about a possible association between Reye's syndrome and aspirin in June 1982.[15] The American Academy of Paediatrics initially endorsed this, but later that year reversed its decision, as did the Secretary of State for Health and Welfare. Thus, at the end of 1982, it was decided not to order the warning labels for all aspirin-containing products that had been considered earlier that year. The reason for these reversals was the controversy regarding the conduct of these studies; this needs to be recognised. This was so intense that the US Public Health Service (PHS) set up a task force to undertake further work to try and overcome the acknowl-

Table II. Reye's syndrome surveillance from 1981 to 2001

Reporting period (August–July)	Total reports from the British Isles	Revised diagnosis (inherited metabolic disorder)	Cases of Reye's syndrome[a]	Number of deaths of cases
1981–2	47	7 (3)	40	26
1982–3	69	10 (6)	59	34
1983–4	93	12 (3)	81	36
1984–5	64	8 (2)	56	32
1985–6	53[b]	13 (4)	39	22
1986–7	47	21 (11)	26	13
1987–8	44	12 (3)	32	19
1988–9	31	13 (6)	18	9
1989–90	24[b]	8 (5)	15	7
1990–1	25	13 (8)	12	5
1991–2	23[c]	6 (5)	15	6
1992–3	21[d]	10 (6)	5	4
1993–4	20[e]	13 (7)	3	3
1994–5	17[c]	3 (2)	12	3
1995–6	18[b]	2 (1)	15	7
1996–7	7	2 (2)	5	4
1997–8	11	4 (2)	7	5
1998–9	11	4 (3)	7	2
1999–2000	4	1 (1)	3	2
2000–April 2001	3	2 (1)	0	0
TOTAL	632	164 (81)	450	239

a Compatible with diagnostic criteria.

b Follow-up not received for one case.

c Follow-up not received for two cases.

d Follow-up not received for five cases and one case did not meet the case definition.

e Follow-up not received for four cases.

edged biases/criticisms inherent in the studies. These have been reviewed in detail and are given in summary form, from which the original publications can be identified and the particular biases acknowledged.[2]

The task force study, which had an initial pilot phase in 1984, attempted this. However, as was pointed out,[16] it still retained a potential for biases. Most important was the widespread knowledge of the Reye's syndrome-aspirin association in the US in 1984, which meant that there could have been preferential diagnosis and/or reporting of patients who fulfilled the criteria whenever there was a history of aspirin ingestion. Some parents may either have reported aspirin usage (perhaps with litigation in mind) or assumed/mistakenly believed it had been given once they were told the diagnosis.

The findings of the first stage of the PHS study had been intended only to establish an appropriate methodology (pilot phase);[8] however, the US Department of Health considered the evidence of such significance that voluntary product labelling was requested in early 1985, and a public service education campaign was immediately initiated. In 1986, legislation requiring product labelling was passed. Thus, it was accepted at that time that there was a case to answer.

The main PHS study followed in 1985–6 and confirmed the earlier findings of a significant association between Reye's syndrome and aspirin usage.[9] Twenty-six of 27 cases (96%) versus 53 of 140 controls (38%) had been so exposed – giving an odds ratio (OR) of 40 and a lower 95% CI of 5.8. Although no major criticisms of the main study subsequently appeared in the literature, there remained some concern about methodology, mainly relating to certain biases which, it was felt, still had not been satisfactorily overcome.

As a result, a further investigation, often known as the 'Yale' study, was conducted by investigators who had not been part of any previous study.[10] Every attempt was made to overcome all earlier biases and the findings of this exacting work yet again confirmed those already published: 21 of 24 cases (88%) compared with 8 of 48 controls (17%) had been exposed to aspirin during the febrile prodrome (OR 35, lower 95% CI 4.2; table I). Subanalyses of the data, addressing the various biases mooted earlier, continued to show that there was indeed a significant association with aspirin.[10] The authors quoted Sir Austin Bradford Hill: "the more anxious we are to prove that a difference between groups is the result of some particular action we have observed, the more exhaustive should be our search for an alternative explanation of how that difference has arisen". They concluded that they had satisfactorily addressed alternative explanations and that the association between aspirin and Reye's syndrome was indeed a real one.

It should be emphasised that there has never been any published criticism of this study. Hence the case for a causal (co-factor) link with aspirin became stronger, although it is worth observing that Orlowski and colleagues[4] barely mention its findings, but merely refer to "several uncontrolled studies from various countries of a purely descriptive nature".

3. British Risk Factor Study

Because of differences between Reye's syndrome epidemiology in Britain and that in the US, it was considered that a descriptive study should be undertaken to generate aetiological hypotheses that might warrant more detailed research. At the beginning of 1984 a 2-year British Risk Factor Study (BRFS) was initiated.[17] Cases ascertained through the BRSSS and parents were interviewed. In addition to satisfying the diagnostic criteria of the surveillance scheme, cases were allotted a 'Reye score' (see paper for details[17]) as a measure of how closely a child's illness conformed to the classical characteristics – clinical, biochemical and pathological – of the North American cases. An individual who was blinded to risk factor exposure carried out the scoring. Analysis of the first year's data (end of 1984) revealed that >50% of the cases had been exposed to aspirin. Hence, a comparison group was recruited for the second year of the study.

The main findings of the BRFS can be summarised as follows: most importantly, there was a highly significant case-comparison excess of aspirin

exposure (63 of 106 [59%] cases vs 48 of 185 [26%] comparisons; $p < 0.0000001$). Analysis of aspirin use in England and Northern Ireland separately was undertaken and showed virtually identical differences; in addition, all six Reye's syndrome cases in Scotland, three of the four cases in the Irish Republic and two of the four cases in Wales had received aspirin. Reye's syndrome cases were also more likely than the comparisons to have had more than one aspirin preparation, and/or to have been given adult formulations. Bearing in mind the inclusive nature of the diagnostic criteria, we think it noteworthy that there was a significant relationship between the height of the Reye score and aspirin exposure (trend $p = 0.0001$); this was in contrast to that for paracetamol (acetaminophen) [$p = 0.07$].

Although this study had important methodological limitations, every attempt was made to address biases such as prior knowledge of the Reye's syndrome-aspirin association. Analysis of Reye score and antipyretic exposure within the 'no prior knowledge' group still showed a highly significant correlation between this score and aspirin (but not paracetamol) exposure. Protopathic bias was also excluded by repeating the case-comparison analysis of aspirin exposure after exclusion of Reye's syndrome cases given the drug on the day of 'onset' or thereafter. 'Onset' was arbitrarily defined as the first day on which anorexia, vomiting, drowsiness or behavioural change occurred – except where the latter was reported within 24 hours of the start of the prodrome. Thus defined, 17 cases were excluded, nevertheless a significant difference remained between the two groups as a whole ($p = 0.004$). These data further support the association between Reye's syndrome and aspirin exposure and, notably, no major findings emerged to contradict the US findings.

4. Resulting Reaction in UK and Political Action

During early 1986, the UK Committee on Safety of Medicines (CSM) reviewed the evidence for an association between Reye's syndrome and aspirin and concluded that public health action should be taken. Accordingly, in mid-June all UK doctors were sent a warning letter advising them not to prescribe aspirin for children <12 years of age, except in certain specific conditions. In addition, a notice appeared in the *British Medical Journal*,[18] there was considerable publicity in the media and warning leaflets were placed in pharmacies and doctors' surgeries. A warning label was required to be placed on all preparations containing aspirin: "do not give to children under 12 unless your doctor tells you to". Moreover, the pharmaceutical industry voluntarily withdrew paediatric aspirin products.

Since April 1998, all aspirin-containing medications sold in the UK have also been required by the Medicines Control Agency to state in the patient information leaflet: "there is a possible association between aspirin and Reye's syndrome when given to children with a fever". However, in April 2002, the CSM issued new advice that aspirin should also be avoided by those aged 12–15 years if patients are feverish.[19] This was because 10 of 17 (59%) aspirin-associated cases reported after 1986 were aged >12 years.[20] The advice was further modified in April 2003 to state: "do not give to children under 16 years unless on the advice of a doctor".[21] Current advice (November 2006) on the Department of Health website mentions the aspirin warning in relation to emergency management of pandemic flu in children and teenagers.[22] From October 2003, packets containing aspirin were required by statute to include this warning label.[21] To my knowledge, 12 other countries – nine in Europe – currently issue some form of public health warning.

5. Evidence for a Causal Relationship

Epidemiology shows only an association between a disease and risk factors, but what about the question of actual causation? In light of standard criteria, I will now summarise the strength of evidence linking aspirin and Reye's syndrome causally.

5.1 Consistent Association on Replication

All six US case-control studies[5-10] (table I) and the BRFS[17] demonstrated a significant association between Reye's syndrome and taking aspirin for

prodromal symptoms. However, two other studies (one in two parts) will now be mentioned that have not demonstrated an association.[23-25]

1. The second study conducted by Orlowski et al.,[24] which was an expansion of the first,[23] had more cases and a control group. Children were in hospital at three centres in Australia (1972–86) and data were obtained by retrospective case-note review. Aspirin exposure was reported in only 8% of cases and 3% of controls and it was concluded that there was no association between aspirin and Reye's syndrome. These studies were criticised on a number of grounds,[26-29] and all the correspondents highlighted the differences between Orlowski's cases and those studied in the US, in particular the young age of the patients. They surmised that his Australian patients mostly did not have 'classic' Reye's syndrome and this was confirmed when the cases were subsequently reviewed using more precise criteria.[30] Orlowski then concluded that none of the original 49 patients could be seen in retrospect as having certain Reye's syndrome, and it was suggested that the absence of 'classic' Reye's syndrome reflected the dearth of aspirin use in Australia in the 1970s, by contrast with that in the US.[29]

2. The second case-control study that was said not to demonstrate an association between aspirin and Reye's syndrome was conducted in Japan.[25] However, details of the methodology were never published in a peer-review journal – thus precluding proper evaluation.

Purely descriptive research such as that from Norway, Spain and other places does little to enhance the case either way and have been summarised recently.[2]

A final piece of evidence supporting the consistency of the association was the finding of an increased risk of Reye's syndrome among children taking long-term salicylate therapy for connective tissue disorders;[31] Moreover, 14 of 361 (4%) cases reported to the national Reye's syndrome surveillance system in the US (1980–97) for whom data on aspirin use were available, were on long-term salicylate medication for connective tissue disorders or Kawasaki disease.[32] This figure should be compared with that of the US annual reported incidence of one case (at most) per 100 000 children <18 years of age; and that was in the years before aspirin warnings.

5.2 Strong Association

All of the analytical epidemiological studies showed highly significant associations between aspirin exposure and Reye's syndrome.[5-9] Although this was reduced on re-analysis of the early US studies, the rigorous method of addressing biases in the Yale study yielded a convincing strength of association.[10]

5.3 Specific Association

Other exogenous agents have been linked with Reye-like illness, for example the antiepileptic drug valproic acid (sodium valproate), aflatoxin in Thailand, bongkrekate in Indonesia, margosa oil in southern Asia, unripe ackee fruit in Jamaica, hopentanate in Japan and insecticides in Canada.[33] However, none of these agents have been demonstrated to have a significant association with this disorder in a case-control study where Reye's syndrome cases fully satisfied diagnostic criteria; nor have they been studied using this methodology.

It appears from the findings of several of the analytical studies, particularly that by Hall and colleagues[17] that a proportion of patients with Reye's syndrome develop the condition without receiving aspirin. However, it is possible that the proportion who were not exposed did not in fact have Reye's syndrome but instead had one of the inherited metabolic disorders or some other condition that can mimic the syndrome. This hypothesis was strengthened by the observation that the higher the 'Reye score' in the British Reye's syndrome cases, the more likely they were to be have been exposed to aspirin (see section 3).

Therefore, it seems likely that Reye's syndrome is a heterogeneity of disorders that might consist of two groups: the first is 'idiopathic' Reye's syndrome (the 'classic' North American type of older cases with a respiratory or varicella prodrome, profuse vomiting and encephalopathy); and the second is a

mix of conditions including the Reye's syndrome-mimicking inherited metabolic disorders, which tend to present in younger patients in whom aspirin use is unlikely to have a role in precipitating encephalopathic episodes. This hypothesis is supported by the findings of a study based on the BRSSS and BPSU data that will be described shortly.[34]

5.4 Dose-Response Relationship

The early clinical studies of Reye's syndrome in which the 'response', measured by severity of the encephalopathy, was apparently related to blood salicylate levels, were later criticised and the results considered unreliable on the grounds that the salicylate methodology was insufficiently precise.[35] Among the US epidemiological studies (in which aspirin exposure data were obtained by parental interview), the PHS pilot study reported that there was no relationship between the daily dose of aspirin taken per unit body weight and the deepest grade of coma attained.[8] Furthermore, there was no difference between the average daily doses taken by cases and controls. However, the PHS main study and the Yale study both demonstrated a significant excess aspirin dosage among cases compared with controls that received aspirin, although neither studied dosage in relation to clinical severity (table I).[9,10]

However, it appears that there is a substantially increased risk of Reye's syndrome even at low dosage. The Yale study demonstrated that even a low total dose (defined as <45 mg/kg) was strongly associated with a risk of Reye's syndrome (OR 20).[10] The PHS main study showed that although cases given aspirin were more likely to have received higher doses than controls also given the drug, three of the 26 Reye's syndrome cases had received <10 mg/kg/day.[9] Cases had a median daily dosage of 26.4 mg/kg, the lowest values being 4.1 mg/kg; figures for the controls were 11.1 and 2.4 mg/kg, respectively. A further analysis of these data concluded that there was a dose-response effect; however, the authors commented that "no safe dose [of aspirin] exists".[36]

We have already commented on the BRFS that found Reye's syndrome cases were more likely to have received adult preparations than comparison cases who had taken the medication.[17] Absence of a consistent dose-response relationship is not incompatible with the concept of aspirin as a causative factor in Reye's syndrome, if its role is idiosyncratic or as an adjuvant or co-factor to an innate susceptibility. As is the case with penicillin hypersensitivity, dosage may be irrelevant.

5.5 Temporal Association

The US case-control studies went to great lengths to demonstrate in each case that aspirin was given before the onset of encephalopathy (defined as the onset of vomiting and/or certain neurological symptoms). Although epidemiologically rigorous, this may not have been necessary because it is possible that aspirin might have an exacerbatory role in aetiology, rather than acting as a prime mover. However, the validity of the case-control studies has been challenged by one author on the basis that medication exposure after the onset of vomiting was not taken into account and that antiemetics may have a causal role in Reye's syndrome.[37,38] These views have been criticised on a number of grounds and have not been widely supported.[38-40]

5.6 Surveillance of Trends

These trends – based on retrospective case reporting – are another aspect of the temporal association between Reye's syndrome and the drug. Only the US, the UK and Ireland have relevant data because of long-term national Reye's syndrome surveillance schemes. Various studies in the US have shown that physician prescribing, drug store purchasing and parental use of children's aspirin for fever have declined considerably since 1981.[41,42] In parallel with this, the annual incidence of Reye's syndrome in the US has also declined. For example in 1980, there were 555 cases reported compared with no more than two per year between 1994 and 1997.[32] One elegant study demonstrated that this decline paralleled exactly the upsurge in publications both in professional and lay literature mention-

ing the link with aspirin, which began in the US in 1980.[43]

Similar trends in the British Isles are more difficult to interpret for which there are various explanations:

- Reporting was initiated more recently than in the US hence longer-term trends are not available to facilitate comparison.
- Numbers of reported cases are smaller.
- The method of case ascertainment was changed from July 1986, the month after the action by the CSM, which had the potential to exert a confounding effect. The introduction of an active reporting scheme via the BPSU in 1986, which would have been expected to enhance Reye's syndrome ascertainment, clearly did not have this effect (as table II illustrates). The most that can be said is that the existing decline levelled out, to be followed in 1996–7 (August–July) by a further substantial decline in case reports; and in the final 6 months to April 2001, by no cases being reported.

In parallel with these trends, a survey of aspirin usage in Northern Ireland showed that children with simple febrile illnesses being admitted to hospital were 17 times less likely to have received aspirin in 1988–9 than in 1985–6.[44] It is notable also that, in spite of an influenza epidemic in the winter of 1989–90, which was the largest since 1976, no accompanying upsurge in the incidence of Reye's syndrome materialised.

6. Evidence for Aspirin-Associated Reye's syndrome

In addition to the decline in incidence since 1986, certain clinical and epidemiological characteristics of reported Reye's syndrome in the UK and Ireland also changed; most notably the median age of patients almost halved. One key study explored the relationship between these qualitative trends and the 1986 aspirin warning.[34] The hypotheses, as stated in section 5.3, were that patients reported to have Reye's syndrome are a heterogeneous group comprising not only the Reye-like inherited metabolic disorders, but also a separate group in which aspirin is the risk factor. The latter would be clinically and epidemiologically distinct and resemble the North American cases. All cases reported to the BRSSS or BPSU (1982–90) were allotted a score (blindly) in which those showing the typical clinical and pathological features of 'classical' (North American) Reye's syndrome, scored highly. The findings showed that high-scoring cases were significantly more likely to have occurred in the 4.5-year period before June 1986 than in the same period afterwards ($p < 0.006$). Among the highest scoring group (17–25) there were 102 pre-warning (35% total that period) and 21 (16%) post-warning. Only two of 28 cases scoring 21–25 occurred after June 1986. Higher-scoring cases were also more likely to have received aspirin ($p < 0.0001$), and to be older than the lower scoring cases ($p < 0.008$).

The study provided further evidence to support the association between aspirin and Reye's syndrome. It also provided a counter argument to those critics who suggest that the decline in Reye's syndrome could be explained by increased recognition of metabolic, viral or toxic diseases.[38,45] If this were the case, all score categories should have declined at equal rates, whereas the decline was significantly greater in the subset of patients who most resembled 'classic' Reye's syndrome. Furthermore, detailed investigations of classical cases of Reye's syndrome in the US and Northern Ireland have not revealed any inherited metabolic disorders[4,46] (JFT Glasgow, unpublished observations in 40 consecutive patients). By virtue of their older age they are also the least likely group to have such disorders. Moreover, anecdotal experience at referral centres also suggests a striking decline in patients with these manifestations.[47,48]

My conclusion from these data is that there is a highly suggestive link between prodromal aspirin usage and Reye's syndrome that is consistent and strong and specific, and that since warnings were issued only occasional cases have occurred despite continued monitoring (table II).[14,32]

7. Experimental Research

Studies are now presented that show there is biological plausibility for the view expressed. These experiments relate to studies of β-oxidation (ketogenesis) in skin fibroblasts from recovered Reye's syndrome patients and normal controls using tritiated palmitate; no work on the response of β-oxidation in human tissue to aspirin metabolites had been carried out previously, nor had the possibility been investigated that cells from Reye's syndrome patients might differ (from those in controls) in their response to these compounds.

Salicylate, the primary metabolite of aspirin, is further metabolised in mitochondria to hydroxyhippurate and gentisate. It is known that salicylate inhibits β-oxidation of medium and long chain fatty acids in rodent liver mitochondria,[49,50] and decreases oxidation of long chain fatty acids by rat liver slices,[51] rat hepatocytes[52] and *in vivo* in mice.[50] The effect on β-oxidation inhibits lactate-driven gluconeogenesis.[52] Moreover, aspirin metabolites have structural similarities to the acyl-portions of the substrate and product of the 3-hydroxyacyl-CoA dehydrogenase activity of the β-oxidation pathway. Therefore, we proposed that aspirin metabolites could be inhibitory at this step, but that this inhibition would not occur in cells lacking this enzyme activity.

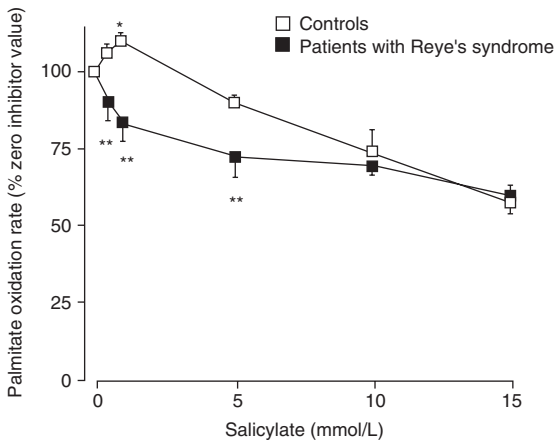

Fig. 1. Salicylate inhibits palmitate β-oxidation differentially in fibroblasts from controls (n = 6) and patients with Reye's syndrome (n = 6). Rates are percentage of the zero value within each independent experiment and are presented as means ± standard error of the mean (SEM). These zero salicylate rates were 68 ± 12 and 72 ± 16 pmol/min/mg ± SEM, respectively, for controls and Reye's syndrome cells. Where the error bars are not visible they fall within the symbol. * $p < 0.01$ (paired t-test) for control rates at 1 mmol/L salicylate (74 ± 12 pmol/min/mg ± SEM) compared with rates at zero salicylate; ** $p < 0.01$ (ANOVA) for Reye's syndrome compared with controls at the same concentration of salicylate.[3]

However, we were unsure whether this would be demonstrable at the serum concentrations reported in the acute phase of Reye's syndrome – namely 0.9 mmol/L (mean salicylate concentration).[53] Levels of hydroxyhippurate tend to be 2-fold higher.[54] Usual doses of the drug that achieve concentrations in the range of 1–5 mmol/L are thought to exert optimum anti-inflammatory effect.[55] As stated at the outset, these data have been published,[3] summarised as a leading article[56] and in a chapter on aspirin and related drugs.[2]

In summary they showed the following:

- Salicylate concentration of <5 mmol/L is a more effective inhibitor in Reye's syndrome cells than in those from controls (figure 1), but in the latter, hydroxyhippurate and gentisate were more inhibitory than salicylate.
- The target for inhibition is long chain hydroxyacyl-CoA dehydrogenase (LCHAD), which is part of the mitochondrial trifunctional enzyme (MTE) of the β-oxidation system.
- Cells from Reye's syndrome patients were significantly more sensitive to inhibition of β-oxidation by salicylate at plasma concentrations within the therapeutic range[55] than are control cells. Actually, in control cells (at 1 mmol/L salicylate), there is significant stimulation of palmitate oxidation, an effect quite opposite to that noted in Reye's syndrome cells (figure 1). The stimulatory effect of salicylate is also present (9–21%) in LCHAD deficient fibroblasts; therefore, stimulatory and inhibitory effects of salicylate are independent and only inhibition requires the presence of LCHAD.

As salicylate is known to uncouple β-oxidation from phosphorylation, the stimulatory effects on the

former in control cells might be due to the presence of an uncoupling protein. Conversely, in Reye's syndrome cells, it is possible that salicylate does not uncouple oxidative phosphorylation because a specific target protein is missing.

We argue that if this innate sensitivity is widely expressed in body tissues, it could explain the action of aspirin on the MTE-LCHAD enzyme system, which in the presence of viral infection may precipitate the encephalopathy.[3,56]

8. Conclusions

There is now a substantial body of evidence that a strong link exists between aspirin usage during the febrile prodrome and 'classic' Reye's syndrome. This goes far beyond a mere epidemiological association. Governments have reacted to these findings in order to prevent the disorder and it sequelae. In those countries where public and professional warnings have been issued, there has been a very striking decline in the number of cases and, as was stated in an editorial of the *New England Journal of Medicine*, "this is nothing short of a public health triumph" in terms of the death and disability avoided.[57] Moreover, as demonstrated in intact human cells, there is now biological plausibility for the role of aspirin and its metabolites as significant inhibitors of LCHAD in mitochondrial function – even at low concentrations of the drug. Such inhibition likely contributes to organelle failure – the key biochemical dysfunction in Reye's syndrome. This occurs in individuals who have an innate, but demonstrable, difference in intermediary metabolism to the effects of the drug at therapeutic levels, which during viral attack acts as a co-factor in precipitating the 'classical' syndrome.

Therefore, it is essential to maintain both public and professional vigilance, especially as the risks of a major influenza outbreak have greatly increased recently. The aim is to ensure that Reye's syndrome will never again become an important cause of mortality and morbidity. Although an occasional voice is raised to dispute the evidence here summarised,[4] prevention is eminently achievable if my argument continues to hold sway.

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