Quantitative Evaluation of the Extent of Hepatic Enzyme Changes in Reye Syndrome Compared with Normal Liver or with Non-Reye Liver Disorders: Objective Criteria for Animal Models

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ABSTRACT. Five enzymes were measured in 50 liver specimens (18 normal liver, 20 Reye liver, 12 diverse liver disorders other than Reye syndrome). The enzymes were: glutamic dehydrogenase (E.C. 1.4.1.3), monoamine oxidase (E.C. 1.4.3.4), lactate dehydrogenase (E.C. 1.1.1.27), D-glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49), catalase (E.C. 1.11.1.6). The Reye syndrome group showed significant decreases in glutamic dehydrogenase (56%) and monoamine oxidase (70%) compared to normal control tissue and these changes were not characteristic of the non-Reye liver disorder group as a whole. Neither catalase nor lactate dehydrogenase appeared to be altered significantly in the Reye or in the abnormal control group compared with normal controls. Thus, only the prominent decreases in the mitochondrial enzyme activities appeared to be highly characteristic of Reye syndrome. Paradoxically, the means of the five hepatic enzymes and the admission levels of two serum enzymes indicative of liver damage (alanine and aspartate aminotransferase) were remarkably similar for both survivors and nonsurvivors of Reye syndrome. (Pediatr Res 19: 19–22, 1985)

Abbreviations

AlaT, alanine aminotransferase
AspT, aspartate aminotransferase
CATAL, catalase
GDH, glutamic dehydrogenase
GPDH, glucose 6-phosphate dehydrogenase
LDH, lactate dehydrogenase
MAO, monoamine oxidase

Twenty years ago Reye et al. (11) in Sydney, Australia, described an encephalography with hepatic involvement as a new and distinct disease entity of childhood. The salient clinical and pathologic features of Reye syndrome have been reviewed previously (1, 2, 4–6, 14). The hepatic changes have been described in detail at the gross and microscopic levels. Panlobular light and ultramicroscopic changes occur uniformly throughout the liver,

without signs of necrosis or biliary stasis. Light and electron microscopic analysis of hepatocytes show an accumulation of microvesicular fat droplets, proliferation of smooth endoplasmic reticulum, glycogen depletion, loss of very low-density lipoprotein particles from the Golgi apparatus, an increase in the number of peroxisomes, and extensive mitochondrial ultrastructural changes. These include mitochondrial swelling with rarification of the matrix, pleomorphism with the appearance of amoeboid-like forms, loss of dense-staining bodies, and reduced succinate dehydrogenase activity as measured by histochemical staining (2). The mitochondrially located urea cycle enzymes (ornithine transcarbamylase and carbamyl phosphate synthetase) also were found to be decreased in activity, prompting suggestions that this deficit could contribute to hyperammonemia (3, 9, 15, 17). To date no comparable evidence for a generalized mitochondrial enzyme deficit has been found in brain or skeletal muscle (12, 13). Either the mitochondrial lesion is unique to the liver, or mitochondrial lesions in other tissues may be focal rather than uniformly distributed throughout the tissue.

The above considerations suggest that a better understanding of the hepatic changes in Reye syndrome, particularly the biochemical alterations, might provide better insights into the origin of the illness and its subsequent progression. Therefore we elected to delineate the biochemical changes in liver with a view to providing a quantitative description of the key features of the disease. Such information should provide objective criteria for evaluating animal models of Reye syndrome.

METHODS

Patient population. Liver specimens were obtained from patients admitted to the Children's Hospital of Michigan, Detroit, MI or to the Children's Hospital, Cincinnati, OH, between 1977 and 1981. Specimens obtained either by needle biopsy or at time of autopsy were obtained with the parents' consent. Liver specimens for the normal infant group were submitted for study by the Office of the Wayne County Medical Examiner. Values for AlaT and AspT were obtained from blood samples taken at the time of admission. For purposes of statistical analysis the cases were assigned to one of three groups (normal liver, Reye syndrome, other types of liver pathology), based on case history, clinical diagnosis, liver pathology, etc.

Assay of enzyme activities in liver tissue. Four enzymes were time assayed by the congruent assay procedure described previously (10). A fifth enzyme, CATAL, was assayed by measuring the first order rate constant for the disappearance of hydrogen

peroxide as followed by decrease in absorption at 240 nm. The five enzymes (LDH, GDH, MAO, CATAL, GPDH) were assayed using an Aminco DW-2 spectrophotometer and specific activity was expressed as units of enzyme activity per milligram of soluble or particulate protein, as appropriate, depending on the location of activity following high-speed centrifugation of the tissue homogenate.

Statistical analysis of data. Enzyme activities measured as above were obtained from 50 human liver specimens. The reproducibility of the assays was tested during the study, using tissue from two normal and two Reye cases, as described in "Results". The significance of differences between means was determined by one-way analysis of variance or by Student's *t* test using either a pooled or separate variance estimate based on the F statistic.

RESULTS

Patient and control admission data. Table 1 summarizes available admission data for 33 cases. No blood chemistry data were available for many of the normal liver control cases where liver tissue was obtained at autopsy within 12 h of unexpected death.

Table 1. *Available admission data for control cases (no liver disorder, group 1), Reye syndrome (group 2), and liver disorders other than Reye syndrome (Group 3)*

ID	Diagnosis	Group	Sex	Age (yr)	AspT (IU/liter)	AlaT (IU/liter)
R705	Reye	2	F	3.9	258	309
R802	Reye	2	M	4.6	1310	1755
R810	Reye	2	F	7.6	960	1050
R811	Reye	2	F	7.2	610	1320
R902	Reye	2	M	9.0	370	760
R907	Reye	2	M	12.2	104	231
3	Recovered Reye	1	F	6.4	42	32
4	Varicella	3	M	13.0	46	52
5	Gastroenteritis, with fatty liver	3	F	6.7	322	230
6	Diarrhea, ear infection	3	F	1.9	742	746
7	Unknown metabolic disorder	3	M	8.1	60	136
8	Hepatitis (mononucleosis)	3	F	9.0	722	784
9	Reye	2	F	11.6	352	528
10	Reye	2	F	2.1	306	406
13	Reye	2	M	14.3	1820	2420
14	Reye	2	M	5.3	866	1680
15	Reye	2	F	3.2	882	740
16	Reye	2	M	1.9	92	52
17	Hepatitis (Epstein-Barr virus)	3	M	13.8	512	686
18	Acetaminophen overdose	3	F	17.0	242	344
19	Encephalitis	1	F	10.3	30	19
20	Reye	2	M	21.4	350	790
24	Reye	2	M	6.3	259	700
25	Reye	2	M	11.5	660	530
26	Reye	2	F	10.6	217	38
27	Reye	2	F	6.9	1400	640
28	Reye	2	M	15.4	340	307
29	Hepatitis	3	F	3.0	820	
30	Reye	2	M	5.2	363	83
34	Unknown	3	M	3.6	302	
36	Urea cycle deficiency	3	M	0.8	35	41
39	Reye	2	M	8.0	240	
41	Methylmalonic aciduria	3	F	0.5		

This control sample contained cases of cardiopulmonary arrest, cerebral hemorrhage, asphyxiation, smoke inhalation, multiple injuries, and sudden infant death syndrome.

Are the enzyme analyses reproducible? Potential sources of error in the analysis of enzyme activities in liver tissue include time-dependent changes during tissue storage in liquid nitrogen, variations in sampling due to nonuniform distribution of activity within the specimen of liver tissue, and errors in measurements of enzyme activity and protein concentration. To assess the overall reproducibility of the assays over the time period of the study (2 yr), the five enzymes were routinely assayed in stored tissue using tissue aliquots from two Reye and two normal liver control specimens. None of the enzymes showed evidence for time-dependent changes over the 2-yr test period, either for the Reye or the control group (Table 2).

Are there significant differences in liver enzyme activities between three predefined groups (normal, Reye, and liver disorders other than Reye)? The five criterion enzymes were measured in 50 liver specimens (18 normal, 20 Reye, and 12 diverse liver disorders other than Reye syndrome). Results are shown in Table 3. For LDH the grand mean of all three groups was 769 units and there were no significant differences between any pair of groups.

For GDH the mean of the Reye group was considerably lower than both the normal and the abnormal control groups and these difference were significant ($p < 0.05$). Nevertheless, the greatest difference was the decrease in Reye group activity compared with normal. With MAO, significant ($p < 0.05$) and striking differences were found when the Reye group was compared with either the normal or the abnormal control groups. By contrast, the small difference between the normal and abnormal control groups was not significant. With CATAL all groups were close to the grand mean, (360 units) and there were no statistically

Table 2. *Reproducibility of enzyme assay procedures**

Case	*n*	Mean	SD	SE	*p*
LDH					
C1	8	775	79	28	} 0.027
C2	8	909	128	45	
R811	7	709	101	38	} <0.001
R902	7	984	92	38	
GDH					
C1	8	113	21	7	} 0.001
C2	8	156	20	7	
R811	7	55	29	11	} NS
R902	7	46	9	4	
MAO					
C1	8	316	62	22	} 0.006
C2	8	424	70	25	
R811	7	167	47	18	} <0.001
R902	7	50	27	10	
CATAL					
C1	8	505	49	17	} 0.004
C2	7	422	43	16	
R811	6	351	51	21	} 0.001
R902	7	227	20	8	
GPDH					
C1	8	51	8	3	} NS
C2	8	46	6	2	
R811	6	65	12	5	} NS
R902	7	76	14	5	

* Specific activities are expressed as: LDH, GDH = (activityy in IU × 10^3) per mg of soluble protein. GPDH = (activity in IU × 10^4) per mg of soluble protein. CATAL = (first order rate constant for hydrogen peroxide loss, in min^{-1} × 10^2) per mg of soluble protein. MAO = (activity in IU × 10^5) per mg of particulate protein.

Table 3. *Group comparisons of liver enzyme activity for Reye and two control groups**

Group	n	Mean	SD	SE	Significant pairs
LDH					
Normal	18	813	232	55	None
Reye	20	770	273	61	
Other	12	703	231	67	
GDH					
Normal	18	131	27	6	a a
Reye	20	58	21	5	aa
Other	12	102	37	11	aa
MAO					
Normal	18	338	84	20	a
Reye	20	105	85	19	aa
Other	12	313	175	50	a
CATAL					
Normal	18	362	88	21	None
Reye	20	352	80	18	
Other	12	365	119	34	
GPDH					
Normal	18	32	13	3	a
Reye	20	46	18	4	
Other	12	67	59	17	a

* Enzyme activities expressed as described for Table 2. Statistically different means are denoted by vertical pairs of letters.

significant differences between any pairs of groups. With GPDH both the Reye and the abnormal control groups were elevated with respect to the Reye group, although only the 2-fold elevation of the non-Reye group was significant.

In summary, the Reye syndrome group showed significant decrease in GDH (56%) and MAO (70%) compared to normal control tissue and these changes were not characteristic of the non-Reye liver disorder group as a whole. Neither CATAL nor LDH appeared to be altered significantly in the Reye or in the abnormal control group compared with normal controls. Moreover, although GPDH appeared to be somewhat elevated with respect to normal tissue in both the Reye and the abnormal control group, the elevation was not found to be significant for Reye syndrome in the population tested. Thus, only the prominent decreases in the mitochondrial enzyme activities appeared to be highly characteristic of Reye syndrome.

Are there statistically significant differences in liver enzyme activities between Reye survivors and nonsurvivors? The decreases in GDH and MAO activities found with the Reye syndrome group appeared to represent a highly characteristic feature of the syndrome. The Reye cases in this study encompassed a wide range of severity, spanning stages I–V at the time the liver specimen was obtained. However, inspection of the enzyme data showed in general a close similarity in mitochondrial enzyme activities between patients biopsied at stage I, and who survived, and stage V nonsurvivors.

To evaluate the effect of altered enzyme activities on outcome, Reye cases were assigned to one of two groups, based on survival. Admission levels of AspT and AlaT also were included, since these activities may indirectly reflect liver impairment. The means for the five hepatic and the two serum enzymes did not differ significantly between survivors and nonsurvivors (Table 4).

DISCUSSION

The results of this study confirm and extend previous reports of selective mitochondrial impairment of liver enzymes. Moreover, a highly reproducible, strictly quantitative assay system was used to measure the activities of a carefully selected group of criterion enzymes in liver specimens from a wide range of cases. The cases included Reye syndrome patients (stages I–V), from infants to adult, who had been admitted to either of two different institutions, and whose diagnosis of Reye syndrome had been confirmed by light and electron microscopic analysis of liver tissue obtained by biopsy or necroscopy. In addition, the study employed two control groups, to permit comparisons of the extent of Reye syndrome changes compared with normal tissue, and to see if changes in mitochondrial enzyme activity are unique to the syndrome. The enzyme activities selected for study included the cytosolic enzymes (LDH, GPDH), the peroxisomal enzyme, CATAL), and two mitochondrial enzymes, GDH and MAO.

Because LDH is a ubiquitous enzyme catalyzing a readily reversible reaction it is not a likely candidate as a key regulatory enzyme, and may serve as a reference enzyme in Reye syndrome (10). Unlike LDH, GPDH is a branch point enzyme that can direct reducing equivalents derived from glucose away from the respiratory chain and toward the NADPH-producing pentose shunt reactions. No significant increase in GPDH activity was found in Reye syndrome, although such an elevation was found for non-Reye liver disorders.

CATAL was not elevated in the Reye syndrome population compared with the control groups. This finding is of interest, since morphologic studies showed an increase in the number of peroxisomes in Reye syndrome liver, prompting the speculation that peroxisomal activity may have increased at the expense of mitochondrial oxidative activity. While this possibility may yet be correct, the present study provides no evidence for a simple relation between morphologic changes and enzyme activity.

The mitochondrial enzymes activities were found to be decreased consistently and markedly in Reye syndrome but not in cases of other liver disorders. The extent of this decrease is comparable to that reported (16) for the mitochondrial enzyme ornithine transcarbamylase, although the decrease in GDH

Table 4. *Comparisons of liver and serum enzyme activities for survivors and nonsurvivors of Reye syndrome**

Group	n	Mean	SD	SE	Significant at $p < 0.05$?
LDH					
Nonsurvivor	12	855	306	88	No
Survivor	8	641	154	54	
GDH					
Nonsurvivor	12	59	20	6	No
Survivor	8	56	23	8	
MAO					
Nonsurvivor	12	100	70	20	No
Survivor	8	111	108	38	
CATAL					
Nonsurvivor	12	331	90	26	No
Survivor	8	384	51	18	
GPDH					
Nonsurvivor	12	45	19	6	No
Survivor	8	46	16	6	
AspT					
Nonsurvivor	12	534	363	105	No
Survivor	8	670	627	222	
AlaT					
Nonsurvivor	11	803	596	180	No
Survivor	8	702	751	265	

* Enzyme activities expressed as described for Table 2.

found here was less than that reported by Robinson *et al.* (13), who found a 90% decrease of activity, based on a wet weight comparison. The reason for this discrepancy is unknown; an approximate estimate of the enzyme content of our specimens on a wet weight basis indicated that the normal control values for GDH as well as previously reported (10) values for glucose 6-phosphatase in Reye and control cases were quite similar to those reported by Robinson *et al.* (13).

The decreased levels of activity may reflect a decreased level of mitochondrial protein. Although attempts were made to distinguish between Reye and control cases by analyzing the readily solubilized protein fraction from Reye and normal liver using a 2-dimensional gel electrophoresis, no consistent pattern of mitochondrial protein depletion could be found because of the complexity of the resulting electrophoretogram (R. A. Mitchell, unpublished results). Decreased activity could result either from impaired mitochondrial biogenesis or from an increased rate of mitochondrial protein turnover. Because mitochondria exist as semiautonomous organelles subject to non-Mendelian genetic defects (7), for example, it is possible that an unknown agent causes an early, transient decrease in the mitochondrial hepatocyte activity, either by impairing mitochondrial biogenesis, or by increasing mitochondrial degradation. It is of interest that only two non-Reye cases (Epstein-Barr virus and giant cell hepatitis) showed mitochondrial enzyme decreases similar to those of Reye cases. Detection of other cases of mitochondrial impairment (*e.g.* by histochemical staining for succinate dehydrogenase activity) may provide useful insights into Reye etiology. An association between Epstein-Barr virus infection and Reye syndrome has been reported (8).

The present study confirms the view that mitochondrial changes in hepatic tissue are highly characteristic of Reye syndrome, although as noted above, not unique to it. It must be emphasized that these findings apply only to liver and cannot be extrapolated to brain or skeletal muscle, where biochemical analysis indicates no decrease in mitochondrial enzyme activity (13). Indeed, abnormally high catabolic activity in muscle, leading to excessive production of ammonia (16), suggests an increased rather than a decreased oxidative metabolism in muscle.

Quantitative analysis of the selected mitochondrial enzymes also confirms the seeming paradox that the severity of the liver damage (as judged by elevation of serum transaminase) is poorly related to outcome. This conclusion is clear from the comparison of enzyme activities between Reye survivors and nonsurvivors. Additional factors appear to be involved in determining the extent of neurologic damage.

In summary, decreased hepatic activity of GDH (56%) and MAO (70%) was highly characteristic of Reye syndrome but not unique to it. Nevertheless, the levels of these mitochondrial enzymes were not significantly different between survivors and nonsurvivors, thereby pointing to additional factors responsible for the severity of the encephalopathy. This suggests that animal models for Reye syndrome should at least show an similar depletion of these activities, possibly associated with highly variable degrees of encephalopathy.

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