Pediat. Res. *12:* 1045–1047 (1978)

Reye's Syndrome: Preservation of Mitochondrial Enzymes in Brain and Muscle Compared with Liver

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Summary

The activities of five mitochondrial enzymes tested in liver from patients with Reye's syndrome were measured. Citrate synthase, glutamic dehydrogenase, succinic dehydrogenase, pyruvate carboxylase, and pyruvate dehydrogenase were all outside of the range shown by control samples and well below them in activity. The activity of two extramitochondrial enzymes, glucose-6-phosphatase, which is a microsomal enzyme, and fructose-1,6-diphosphatase, which is a soluble enzyme, were in the normal range in samples from Reye's syndrome patients. In both muscle and brain the activities of the mitochondrial enzyme, citrate synthase, glutamic dehydrogenase, and succinic dehydrogenase were all within the control range. Pyruvate dehydrogenase was found to be normal in muscle from these patients.

Speculation

Enzymatic changes in mitochondria associated with Reye's syndrome differ in liver, brain, and muscle, whereas mitochondrial structural alterations appear to be similar in these tissues.

Increasing evidence suggests that enzyme deficiencies localized in liver mitochondria are widespread in Reye's syndrome and involve not only the urea cycle (2, 12, 13, 16) but also pyruvate dehydrogenase (9), pyruvate carboxylase (9), and succinic dehydrogenase (1). It has been suggested from microscopic observations on brain and muscle biopsies of children with Reye's syndrome that ultrastructural changes seen in hepatic mitochondria (6) also are present in brain and muscle mitochondria (5, 7). In this communication we examine from a biochemical standpoint the status of mitochondrial enzymes in liver, muscle, and brain, and conclude that the primary site of mitochondrial pathology is the liver.

CASE MATERIAL

Postmortem tissue from five patients with Reye's syndrome was studied together with tissue from control subjects. The group studied consisted of two females and three males, all of whom demonstrated the classic prodromal illness followed by confusion, drowsiness, and rapid onset of encephalopathy. The time lapse between onset of encephalopathy and death was 3 or 4 days in every case studied, as reported previously. In addition, they all showed elevated serum transaminase and blood ammonia (9). In all five patients the diagnosis of Reye's syndrome was confirmed by the demonstration of characteristic diffuse small droplet fatty infiltration of the liver, swollen mitochondria with loss of cristae, and absence of centrilobular necrosis. Control tissues used were obtained from nine individuals at autopsy, all less than 10 years of age. Five died after a chronic course of debility secondary to congenital heart disease, the other four died acutely of undetermined causes.

MATERIALS AND METHODS

Pieces of skeletal muscle, liver, and brain (cerebrum) were obtained at autopsy after informed parental consent within 2 hr of death, rapidly frozen in liquid nitrogen, and stored at −70° until used for enzyme assay. For enzyme assays listed below, small pieces of tissue were thawed, homogenized in 10 vol ice-cold 0.25 M sucrose, 5 mM Tris-HCl buffer, pH 7.4, and centrifuged at 500 g for 10 min to remove cell debris.

Glucose-6-phosphatase was measured by the method of Swanson (14), fructose 1,6-diphosphatase by the method of Pontremoli

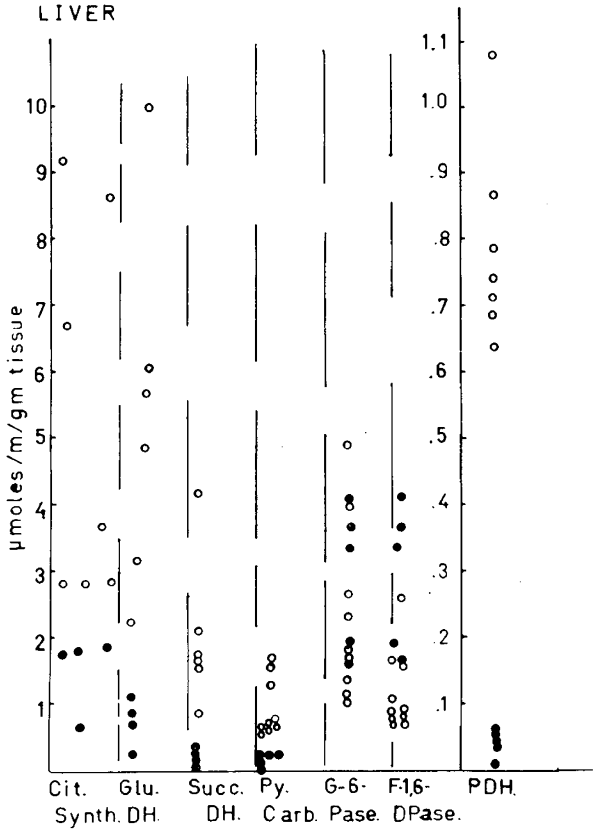

Fig. 1. Activities of hepatic enzymes in postmortem liver from patients with Reye's syndrome (●) compared with control subjects (○). Activities are expressed as micromoles per min per g wet wt tissue. Cit. Synth.: citrate synthase; Glu. DH.: glutamate dehydrogenase; Succ. DH: succinate dehyrogenase; Py. Carb: pyruvate carboxylase; G-6-Pase: glucose-6-phosphatase; F-1,6-DPase: fructose-1,6,-diphosphatase; PDH: pyruvate dehydrogenase.

Table 1. *Enzyme levels in liver of patients with Reye's syndrome*[1]

	Enzyme level (μmol/g liver/min)							
	Patients					Controls		
	1	2	3	4	5	Mean ± SE	Mean ± SE	*P*
Citrate synthase	1.89	1.88	0.66	1.94		1.59 ± 0.31	5.2 ± 1.1 (5)	<0.05[2]
Glutamate dehydrogenase	1.21	0.86	0.15	0.76		0.74 ± 0.22	5.4 ± 1.1 (6)	<0.01[2]
Succinate dehydrogenase	0.13	0.24	0.38	0.32		0.26 ± 0.05	1.95 ± 0.48(5)	<0.025[2]
Pyruvate dehydrogenase	0.011	0.055	0.048	0.041	0.056	0.041 ± 0.008	0.77 ± 0.10 (7)	<0.005[2]
Pyruvate carboxylase	0.097	0.22	0.24	0.23	0.004	0.158 ± 0.046	0.91 ± 0.16 (9)	<0.001[2]
Glucose-6-phosphatase	1.98	4.10	3.67	3.40	1.62	2.95 ± 0.48	2.55 ± 0.52 (8)	<0.30
Fructose-1,6-diphosphatase	1.95	0.62	2.44	2.25	0.59	1.57 ± 0.40	1.40 ± 0.34 (9)	<0.40

[1] Results are expressed as the mean ± SE. Number of controls is given in parentheses. *P* (significance) values are given for each enzyme measured.
[2] Denotes statistically significant differences as indicated by applying Student *t*-test to values obtained.

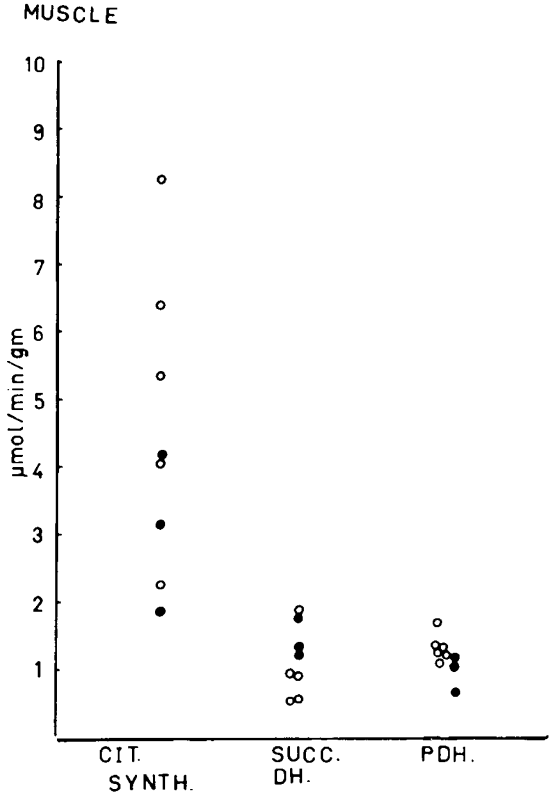

Fig. 2. Activities of enzymes in postmortem skeletal muscle from patients with Reye's syndrome (●) compared with control subjects (○). Activities are expressed as micromoles per min per g wet wt tissue. Cit. Synth.: citrate synthase; Succ. DH: succinate dehydrogenase; PDH: pyruvate dehydrogenase.

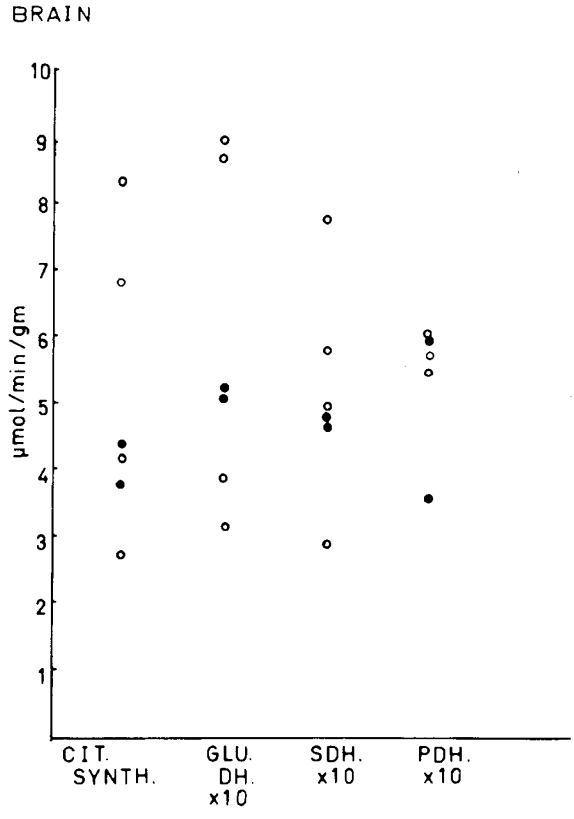

Fig. 3. Activities of enzymes in postmortem brain from patients with Reye's syndrome (●) compared with control subjects (○). Activities are expressed as micromoles per min per g wet wt tissue. Cit. Synth.: citrate synthase; Glu. DH: glutamate dehydrogenase; SDH: succinate dehydrogenase; PDH: pyruvate dehydrogenase.

(8), pyruvate carboxylase by the method of Crabtree *et al.* (3), succinate dehydrogenase by the method of Veeger *et al.* (17), citrate synthase by the method of Shepherd and Garland (11), and glutamic dehydrogenase by the method of Schmidt (10). Assay of whole tissue pyruvate dehydrogenase was carried out as described by Taylor *et al.* (15) using 300–400 mg freshly thawed tissue, homogenized in 10 vol ice-cold buffer containing 10 mM potassium phosphate, 1 mM EDTA, 1 mM dithiothreitol, and 1% fatty acid-free bovine serum albumin, pH 7.4.

RESULTS

The activities of the five mitochondrial enzymes tested in liver from patients with Reye's syndrome, citrate synthase, glutamic dehydrogenase, succinic dehydrogenase, pyruvate carboxylase and pyruvate dehydrogenase, were all outside of the range shown by control samples and well below them in activity. Pyruvate dehydrogenase was particularly badly affected, the highest value being only some 12% of normal activity. However, activity of two extramitochondrial enzymes, glucose-6-phosphatase, which is a microsomal enzyme, and fructose-1,6-diphosphatase, which is a soluble enzyme, were in the normal range in samples from Reye's syndrome patients (Fig. 1). These results are treated statistically in Table 1, the difference in activity being statistically different only for mitochondrial enzymes. In muscle, however, all three enzymes that were measured were within the normal range (Fig. 2) (glutamate dehydrogenase has extremely low activity within that tissue). In brain, of which only two were in a good state of preservation at autopsy since all five patients died of brain-related causes, the activities of citrate synthase, glutamic dehydrogenase, and succinate dehydrogenase were all within the control range

(Fig. 3). Pyruvate dehydrogenase was at normal levels in one brain but somewhat below normal in the other, but certainly not down to 12% of the control value.

DISCUSSION

Ultrastructural changes in hepatocyte mitochondria suggest that a decrease in mitochondrial function may be one of the early events in Reye's syndrome (6). Whether this structural change is confined to the hepatocyte or is also seen in other tissues such as brain and muscle is at the moment a subject of discussion (5, 7). Partin (7) has suggested that brain and muscle mitochondria may be subject to the same pathologic process as liver mitochondria in the course of the disease. Demonstration of enzyme deficiencies in the liver both by ourselves (9) and others (1, 2, 4, 12, 13, 16) points to deterioration of enzyme activity in the mitochondria, although cytoplasmic enzymes are little affected. These results, shown in Figure 1 and Table 1, confirm this on a wide scale without a single exception in any of the enzymes in the five patients' studied. However, both brain and muscle mitochondria do not appear to show the enzyme deficiencies so easily demonstrated in liver. We therefore conclude that the early events in the disease process that result in the decrease in activity of hepatic mitochondrial enzymes do not take place either in muscle or brain. We propose that mitochondrial damage seen in brain and muscle cells of patients with Reye's syndrome is either a secondary phenomenon resulting from the severity of the metabolic disturbance or that it is representative of the same primary process that occurs in liver mitochondria but without the same biochemical sequelae. Despite this severe mitochondrial insult, cellular ATP in the liver appears to be maintained at normal levels (4). This indicates that the liver can supply its energy needs by glycolysis, a cytoplasmic function that remains intact during the course of the disease.

CONCLUSION

We have presented further evidence that the insult that occurs to the liver in Reye's syndrome results in a generalized deletion of mitochondrial enzyme activity. Preliminary data on a small number of patients suggests that this generalized deletion does not occur in other tissues such as brain and muscle.

0031-3998/78/1211-1045$02.00/0

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18. This research was supported by the Weston Foundation and the Medical Research Council of Canada (MRC Grant MA5473).
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20. Received for publication May 10, 1977.
21. Accepted for publication February 15, 1978.

Printed in U. S. A.