Histiocytoid Cardiomyopathy of Infancy: Deficiency of Reducible Cytochrome *b* in Heart Mitochondria

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Summary

A 3-week-old girl with failure to thrive and cardiomegaly died of cardiac arrest at age 4 weeks. Morphologic studies of the heart showed enlarged muscle fibers with large accumulations of mitochondria, characteristic of histiocytoid cardiomyopathy. Biochemical studies showed markedly decreased succinate-cytochrome *c* reductase and rotenone-sensitive NADH-cytochrome *c* reductase activities, while other mitochondrial enzymes were normal. In isolated mitochondria, cytochrome spectra showed a severe defect of reducible cytochrome *b* and a less marked defect of cytochrome *cc*₁, while the content of cytochrome *aa*₃ (cytochrome *c* oxidase) was normal. Histiocytoid cardiomyopathy appears to be due to a defect of complex III (reduced coenzyme Q-cytochrome *c* reductase) in the respiratory chain of heart mitochondria.

"Histiocytoid" cardiomyopathy (also called isolated cardiac lipidosis, infantile xanthomatous cardiomyopathy, lipid histiocytosis, focal myocardial degeneration, or focal lipid cardiomyopathy) is a rare but distinct clinicopathologic syndrome of infancy. It is characterized by severe, recurrent, and fatal arrhythmias or cardiac arrest and by the presence of uniquely altered cardiac cells (11). The abnormal cells are often found in clusters throughout the myocardium, lack myofibrils, and have foamy cytoplasm. By electron microscopy, they show massive accumulation of mitochondria and less marked deposits of lipid droplets and glycogen (1, 4, 11, 28). The disorder has been reported in 18 patients; 15 were girls. All but one case were sporadic, and the etiology is unknown (1, 4, 11, 28).

We have studied an infant girl with typical clinical and pathologic features of histiocytoid cardiomyopathy. Biochemical studies of cardiac muscle showed a marked defect of reducible cytochrome *b*, suggesting that the disease may be due to an inborn error of mitochondrial electron transport.

CASE REPORT

A 3-week-old girl was referred to the Children's Hospital of Los Angeles for evaluation of failure to thrive and tachypnea. She was born at full term gestation to a 17-year-old gravida-2, para-1 mother with "borderline" diabetes. The pregnancy was complicated by urinary tract infections, and the mother took ritodrine hydrochloride from the 6th to the 8th month of gestation (10 mg three times a day during the 6th month, and 10 mg twice a day during the 7th month) to arrest premature labor. The patient's sibling was alive and well at age 3 years.

The baby was well at birth and was discharged in apparent good health. However, she was a poor eater and at 3 weeks of age she weighed 150 g less than at birth. At admission, the patient was alert, with normal spontaneous movements and no evidence of hypotonia. On physical examination there were no significant murmurs; the liver was palpable 3 cm below the right costal margin and the spleen tip was palpable. The chest roentgenogram showed massive cardiomegaly. The electrocardiogram (Fig. 1) showed a short PR interval, left ventricular hypertrophy, and changes suggestive of ischemia. The echocardiogram (Fig. 2) demonstrated hypertrophy of both ventricles and the ventricular septum with diminished contractility consistent with hypertrophic cardiomyopathy. The initial diagnosis was glycogenosis type II (Pompe disease) for which a muscle biopsy was performed. Histochemical examination, including modified Gomori trichrome stain (10), and ultrastructural studies showed no abnormalities. Five days after admission, the baby vomited, became cyanotic, and had cardiac arrest. Cardiopulmonary resuscitation procedures were unsuccessful and the baby died at the age of 4 weeks.

Autopsy was performed, with informed parental consent, 2 h after death. The heart was greatly enlarged and weighed 65 g (expected weight, 20 g). The lungs were atelectatic; liver and spleen were not enlarged. There were multiple cysts in the ovary and hyperplasia of mesenteric lymph nodes. Microscopic studies showed diffuse but focal myocardial changes characterized by enlarged muscle fibers (histiocyte-like cells) with only remnants of myofibrils, large accumulations of mitochondria, slightly increased amount of fat and decreased glycogen content (Fig. 3). Other organs showed nonspecific changes: chronic pneumonitis, mild fatty degeneration of the liver, acute tubular necrosis of the kidney, and multiple follicular cysts of the ovaries.

SPECIAL STUDY METHODS

The muscle biopsy and portions (approximately 2 g) of heart, liver, and kidney obtained at autopsy were stored at -70°C and

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Fig. 1. The admission electrocardiogram (retouched for photographic clarity) shows a short PR interval of 0.06 sec, left ventricular hypertrophy and ST-T wave changes suggestive of myocardial ischemia. An alternative interpretation is that of preexcitation of the ventricles.

Fig. 2. The admission 2-dimensional echocardiogram in the 2-chamber view demonstrates markedly thickened left ventricular walls and a moderate amount of posterior pericardial effusion. LVPW, left ventricular posterior wall; IVS, interventricular septum; PE, pericardial effusion; LA, left atrium.

shipped to the biochemistry laboratory on dry ice. For studies of mitochondrial enzymes, tissues were homogenized in 9 volumes of 0.15 M KCl, 50 mM Tris-HCl (pH 7.4) with all-glass, motor-driven homogenizers, and centrifuged at 1000g for 10 min.

Mitochondria were isolated from cardiac muscle (1–1.5 g) as

described by Bookelman *et al.* (3) for frozen skeletal muscle. Electron microscopic analysis showed that the fraction was composed almost exclusively of mitochondria or mitochondrial fragments. The activities of cytochrome *c* oxidase (27), succinate-cytochrome *c* reductase (22), and NADH-cytochrome *c* reductase

Fig. 3. Electron micrograph of a myocardial cell containing only a few contractile elements. The fibers largely consist of mitochondria showing postmortem changes and large lipid droplets. Uranyl acetate and lead citrate stain. $\times 5700$.

Fig. 4. Schematic representation of the respiratory chain [reproduced from Tzagoloff (25)].

(22) and carnitine acetyltransferase (15) were measured by described spectrophotometric procedures, and the activities of carnitine octanoyltransferase (2) and carnitine palmitoyltransferase by "isotope exchange" assays (20) both in crude tissue extracts and in mitochondrial preparations.

The spectra of reduced-minus-oxidized cytochromes were recorded at room temperature in a double-beam spectrophotometer (Cary 210) as described (3). Respiratory chain components were reduced by the addition of succinate (10 mM) and ADP (1.6 mM) in the presence of cyanide (1.0 mM). To reduce cytochrome *b* selectively, antimycin A, which blocks electron transport between cytochromes *b* and *c*₁ was used instead of cyanide (Fig. 4). The contents of cytochromes were calculated from the height of the peaks using the extinction coefficients suggested by Bookelman *et al.* (3).

Control hearts were obtained at autopsies performed 2 to 4 h after death from five patients (6 months to 68 years of age) who

had died of neurologic or neuromuscular diseases not affecting the heart. To control for postmortem changes, a portion of papillary muscle was obtained during open heart surgery for mitral valve replacement and frozen immediately. The patient was a young adult with chronic hypoxia but without cyanosis. More detailed clinical data are not available, but the cardiac muscle appeared normal by light microscopy. This same muscle was used to study myosin (ATPase activity and subunit composition) and several glycogenolytic and glycolytic enzymes: all data were normal. All tissues were stored frozen in liquid nitrogen or in a deep-freezer at -70°C for periods of 6 months to 5 years.

SPECIAL STUDY RESULTS

In crude extracts of the patient's heart, several mitochondrial enzymes had normal or increased activities, including cytochrome *c* oxidase and the long, medium and short chain acyl-

Table 1. Mitochondrial enzymes in human heart extracts

	Cytochrome <i>c</i> oxidase	Succinate-cytochrome <i>c</i> reductase	SDH	CPT	COT	CAT
Controls (autopsy)	3.89 ± 1.00 (5)	1.48 ± 0.46 (4)	2.22 ± 0.68 (6)	303.7 ± 32.0 (4)	191.6 ± 39.8 (4)	1.58 ± 0.39 (4)
Range	(2.81–5.40)	(1.18–2.16)	(1.14–3.24)	(274.2–346.7)	(154.1–241.8)	(1.03–1.90)
Control (biopsy)	5.83	1.18	1.71	213.7	199.8	
Patient	3.02	0.09	1.14	403.3	290.9	2.90

* For cytochrome *c* oxidase, succinate-cytochrome *c* reductase, succinate dehydrogenase (SDH), and carnitine acetyltransferase (CAT), values are expressed as micromoles of substrate utilized/min/g fresh tissue. For carnitine palmitoyltransferase (CPT) and carnitine octanoyltransferase (COT), values are nanomoles of substrate utilized/min/g fresh tissue. Control values ± SD. Numbers of controls are in parentheses.

Table 2. Enzyme activities in mitochondria isolated from frozen human hearts (see "Materials and Methods")

	Cytochrome <i>c</i> oxidase	Succinate- cytochrome <i>c</i> reductase	NADH-cytochrome <i>c</i> reductase	
			–Rotenone	+Rotenone
Controls	340 ± 75 (4)	197 (3)	480 (3)	315 (3)
Range	(257–405)	(154–236)	(370–540)	(270–378)
Patient	228	25	370	370

* Values are expressed as nanomoles cytochrome *c* oxidized or reduced/min/mg protein ± SD. Numbers of control preparations are in parentheses.

carnitine transferases (Table 1). In contrast, succinate-cytochrome *c* reductase activity was only 6% of the normal mean. This decrease could not be attributed to postmortem changes because the activity was similar in four control hearts obtained at autopsy and in a specimen of cardiac muscle obtained during open heart surgery (Table 1).

The defect of succinate-cytochrome *c* reductase was confirmed in isolated heart mitochondria (Table 2). In the mitochondrial fraction, NADH-cytochrome *c* reductase activity was present but, in contrast to normal, it was insensitive to rotenone (Table 2). The lack of rotenone-sensitive NADH-cytochrome *c* reductase suggested that the defect involved that portion of the respiratory chain which carries electrons derived from the oxidation of both NADH and succinate (Fig. 4). This portion includes complex III (reduced coenzyme Q-cytochrome *c* reductase), cytochrome *c*, and complex IV (cytochrome *c* oxidase) (Fig. 4). Studies of reduced-minus-oxidized cytochrome spectra in isolated mitochondria showed a virtual lack of the cytochrome *b* peak in the α region (562 nm) and, even more conspicuously, in the γ region (427 nm) of the spectrum (Fig. 5). Cytochrome *aa*₃ was normal, as predicted by the normal cytochrome *c* oxidase activity, while the content of cytochrome *cc*₁ was decreased, but to a much lesser extent than cytochrome *b* (Table 3).

Addition of antimycin A to reduce cytochrome *b* selectively revealed a single peak in both the α and γ regions in normal heart mitochondria, while virtually no peak was seen in the patient's mitochondria (data not shown). The activities of cytochrome *c* oxidase, succinate-cytochrome *c* reductase, and NADH-cytochrome *c* reductase ($\pm$ rotenone) were normal in the patient's muscle biopsy (succinate-cytochrome *c* reductase: 0.54 μ mol cytochrome *c* reduced/min/g tissue; mean $\pm$ SD of 17 controls, 1.16 $\pm$ 0.47), and autopsy liver (succinate-cytochrome *c* reductase: 0.97 μ mol/min/g; range of activity for eight controls, 0.64–2.02).

DISCUSSION

The diagnosis of histiocytoid cardiomyopathy in this patient was based on the characteristic pathology of the myocardium. The clinical picture of intractable and rapidly fatal cardiomyopathy in infancy was also similar to those of the other 18 reported cases. The spectrum and significance of the clinical and

pathological changes in this disorder have been reviewed by Ferrans *et al.* (11). The pathologic hallmark of the disease is the presence of severely altered myocardial cells in which myofibrils have virtually disappeared and have been replaced by massive accumulation of mitochondria and a less severe accumulation of lipid droplets (Fig. 1). Although the attention of pathologists has focused on the peculiar shape of these histiocyte-like cells and their increased lipid content, the increased number of mitochondria represents the distinctive and probably primary change (11). However, no biochemical studies have been performed so far and, in fact, an inborn error of metabolism has been considered unlikely (1, 4, 11, 28).

Similar morphologic changes, with greatly increased number of mitochondria and less marked accumulation of neutral fat and glycogen, have been described in skeletal muscle of many patients with "mitochondrial myopathies" (12–15). Systematic studies of muscle mitochondrial function in these clinically heterogeneous disorders have documented an increasing number of specific biochemical errors, including several distinct defects of the respiratory chain components (5, 7, 8, 17).

Application of similar biochemical techniques to the heart of this patient showed severe impairment of the succinate-cytochrome *c* reductase and of the rotenone-sensitive NADH-cytochrome *c*-reductase activities while other mitochondrial enzymes, including cytochrome *c* oxidase, were normal. Analysis of the spectra and content of cytochromes in isolated mitochondria further defined the defect: there was a marked decrease of reducible cytochrome *b* and a mild deficiency of cytochrome *cc*₁. The patient's heart was frozen 2 h post mortem and autolysis may have contributed to the abnormal data. However, this is unlikely for several reasons: 1) control hearts also obtained and frozen 2 h after death did not show similar alterations; 2) the biochemical values in control postmortem hearts were similar to those obtained in freshly frozen cardiac tissue; 3) the biochemical abnormality was specific and other enzymes were not affected.

This presumably inborn impairment of electron transport and energy production could cause mitochondrial proliferation in the heart as it does in skeletal muscle, although the mechanism of this phenomenon remains obscure. A secondary impairment of lipid oxidation could explain the abnormal accumulation of lipid droplets in the hearts of patients with histiocytoid cardiomyopathy.

Cytochrome *b* deficiency in skeletal muscle has been reported in five patients (6, 14, 16, 18, 23) with different clinical syndromes. A father and son (23) and one other patient (18) had limb weakness, dementia, ataxia, myoclonus, and areflexia, while the other patients (6, 14, 16) had only extreme intolerance to exercise. The three unrelated patients had no family history of similar disorders. Cytochrome *b* is part of a complex (reduced coenzyme Q-cytochrome *c* reductase, or complex III) composed of multiple polypeptides. The apoenzyme of cytochrome *b* is one of the few proteins known to be coded for by mitochondrial DNA, while the other components of complex III are encoded in nuclear DNA and synthesized in the cytoplasm (25). Genetic defects of complex III could, therefore, be due to mutations of the mitochondrial or nuclear genome. A mutation of the mitochondrial genome in histiocytoid cardiomyopathy could cause

Fig. 5. Difference spectra of reduced-minus-oxidized cytochromes recorded at room temperature in mitochondrial fractions isolated from frozen heart. Dotted line, control; solid line, patient with histiocytoid cardiomyopathy. The three arrows indicate the peaks for cytochrome aa_3 (602 nm), b (562 nm), and cc_1 (554 nm) in the α region of the spectrum. The lack of the cytochrome b peak in the patient's mitochondria is even more evident in the γ region of the spectrum where a single high peak at 441 nm (cytochrome aa_3) is seen instead of the usual distinct peaks at 427 nm (cytochrome b) and 441 nm.

Table 3. Content of cytochromes in mitochondria isolated from frozen human heart*

	Cytochromes		
	b	cc_1	aa_3
Controls (4)	496 $\pm$ 63	525 $\pm$ 102	705 $\pm$ 52
Range	(419–577)	(428–638)	(643–775)
Patient	16	139	660

* Values are expressed as picomoles/mg mitochondrial protein.

cytochrome b deficiency and explain the lack of evidence for mendelian transmission of the disease. A strictly maternal, vertical type of heredity would be expected for a mitochondrial genetic trait (25) and has recently been shown in several families with "mitochondrial cytopathy" (9), but the invariably fatal nature of histiocytoid cardiomyopathy might prevent this type of transmission. Also, genetic defects of mitochondrial DNA would be expected to be generalized, while in our patient there was no clinical or pathologic involvement of tissues other than the heart, and biochemical findings were normal in skeletal muscle and liver. Conversely, in the four patients with documented defects of reducible cytochrome b in skeletal muscle, there was no clinical or laboratory evidence of cardiopathy (6, 14, 16, 18, 23).

Genetic defects of one or more of the nuclearly coded polypeptides of complex III may impair the assembly of the complex and result in lack of reducible and spectrophotometrically demonstrable cytochrome b . The partial defect of cytochrome cc_1 in our case may be due to a deficiency of cytochrome c_1 , which is also a component of complex III (Fig. 4). Immunological studies have shown lack of at least three complex III proteins in skeletal muscle mitochondria from one patient with a defect of reducible cytochrome b (6, 14). Similar analysis is needed to define the biochemical defect of histiocytoid cardiomyopathy at the molecular level. If the genetic defect involves one or more of the nuclearly coded polypeptides, it should be transmitted by mendelian inheritance for which, however, there is no evidence.

More detailed genetic considerations will have to await definition of the molecular defect. With our patient, 16 of 19 known cases affected girls: this striking predominance of affected females is unexplained.

Several inborn errors of metabolism cause cardiomyopathy: among the glycogenoses, typically infantile acid maltase deficiency (Pompe disease) and, less frequently, debrancher enzyme deficiency (8). Among the disorders of lipid metabolism, systemic carnitine deficiency may cause severe but treatable heart disease (24, 26). Histiocytoid cardiomyopathy represents the first biochemically documented disorder of cardiac mitochondria. Other cardiomyopathies of unknown etiology may be due to specific defects of mitochondrial metabolism, especially when they are accompanied by morphological alterations of mitochondria (13, 19, 21). As in the case of "mitochondrial myopathies," this promises to be a fruitful area of clinical investigation.

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Pulmonary Function of the Reserpine and Isoproterenol Models of Cystic Fibrosis

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Summary

Two experimental animal models exhibiting functional and morphologic changes of exocrine glands similar to those seen in patients with cystic fibrosis (CF) have been reported in the rat: chronic stimulation with reserpine (Martinez *et al.* 1973 *Pediatr Res* 9:463, 470) and chronic stimulation with isoprenaline (Sturgess and Reid 1973 *Br. J. Exp. Pathol.* 54:388). We have studied the pulmonary function of these models induced by injecting rats subcutaneously with reserpine (RES, 0.5 mg/kg/day), isoproterenol (ISO, 25 mg/kg/day), or saline (Con, 1.0 ml/kg/day) for 6 days. Plethysmographic measurements were made for functional residual capacity (FRC), airways resistance (R_{aw}), specific airways conductance (sG_{aw}), phase difference between air flow rate and mean alveolar pressure (PD), frequency of breathing (f), and tidal volume (V_T) of the anesthetized rats. In the RES and ISO rats, the FRC, R_{aw} and f were not different from Con values. The PD was greater and the V_T was less than Con values ($p < 0.05$). The results of both studies indicate uneven ventilation (increased

PD) and *penduluft* (decreased V_T) consistent with maldistribution of resistance and/or compliances of the peripheral airways and/or alveolar compartments. These physiologic effects can be related to the morphologic changes reported for the airways of rats under chronic adrenergic stimulation (ISO) and expected for rats under chronic catecholamines depletion (RES). Since peripheral airways involvement is usually the earliest pulmonary lesion found in CF, these studies indicate that the RES and ISO models may be representative of the early pulmonary involvement of CF.

Abbreviations

CF, cystic fibrosis
RES, reserpine
ISO, isoproterenol sulfate
Con, saline

CF is a genetic exocrinopathy which affects 1:1600 live births among Caucasians. Patients with CF presumably have morphologically normal lungs at birth, as seen in infants dying of meconium ileus (2). The earliest pulmonary lesions in CF are dilatation and hypertrophy of bronchial glands and goblet cells, as well as metaplasia of the bronchiolar epithelium followed shortly by mucous plugging of peripheral airways (17). Subsequently, a vicious cycle of obstruction, chronic infection, and more tissue damage ensues.

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