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Muscle Carnitine Palmityltransferase Deficiency and Myoglobinuria

Abstract. *Muscle carnitine palmityltransferase activity, measured by three different methods, was very low (0 to 20 percent of controls) in a patient with a familial syndrome of recurrent myoglobinuria. Long-chain fatty acyl CoA synthetase activity was normal; acetylcarnitine transferase activity was decreased by 40 percent, and carnitine content was 1.7 times higher than the mean control value. Utilization of palmitate by isolated mitochondria was more impaired than utilization of palmitylcarnitine, suggesting a more severe defect of carnitine palmityltransferase I than transferase II. Thus, myoglobinuria may be due to a genetic defect of lipid metabolism in skeletal muscle.*

In 1970 Engel *et al.* (*1*) described a pair of identical twin girls, 18 years old, with intermittent "cramps" and myoglobinuria, frequently but not always related to exercise. The symptoms were also induced by fasting or a high fat diet. On the basis of metabolic studies, they suggested a defect in the utilization of long-chain fatty acids by skeletal muscle. Long-chain fatty acyl coenzyme A (CoA) synthetase was present in the muscle of these patients, but the activity of carnitine palmityltransferase was not investigated. We now report a defect of carnitine palmityltransferase in muscle of a patient with similar symptoms.

A 29-year old man had episodic muscle "cramps" and pigmenturia for 16 years. The symptoms were often, but not always, related to physical exertion and generally followed the exercise by several hours. Prolonged rather than strenuous exercise appeared to cause symptoms. The urinary pigment was identified as myoglobin on several occasions, and one attack was accompanied by renal shutdown. The patient's only brother had identical symptoms. Between attacks examination was normal. Ischemic work was followed by a normal rise of venous lactate, and contracture was not induced. Ultrastructural study of muscle showed no remarkable abnormality; there was no accumulation of lipid or glycogen. The clinical and morphological investigations have been described (*2*).

To exclude glycogen storage diseases causing myoglobinuria, glycogen metabolism was investigated in a muscle biopsy. The glycogen content was 1.15 percent [1.08 ± 0.04 (S.E.) in 33 controls]; phosphorylase and phosphofructokinase activities were normal.

Carnitine content, measured in frozen muscle (*3*), was 3.25 μmole per gram of tissue. In 13 controls, carnitine concentration was 1.92 ± 0.18 μmole per gram of tissue. The activity of the long-chain fatty acid activating enzyme, palmityl-CoA synthetase, measured in homogenates of frozen muscle (*4*), was normal (Table 1).

The short-chain fatty acyl carnitine transferase, or acetylcarnitine transferase, was determined in muscle extracts by a spectrophotometric method (*5*). Protein concentration was measured by the procedure of Lowry *et al.* (*6*). Acetylcarnitine transferase was about 40 percent less active in the patient's muscle than in six controls (Table 1). Carnitine palmityltransferase was measured by three different methods: (i) in the direction of palmityl-CoA formation (hydroxamate method) (*7*); (ii) by the "isotope exchange" method (*8*); and (iii) by a "forward reaction," in the direction of palmitylcarnitine formation, with DL-[^{14}C]carnitine and palmityl-CoA as substrates (*9*).

Carnitine palmityltransferase was studied in both crude muscle extracts and in mitochondrial fractions isolated from frozen muscle (*10*). In view of the predominant or exclusive mitochondrial localization of carnitine palmityltransferase (*11*), we isolated mitochondrial fractions to increase the sensitivity of the assay. Mitochondrial preparations from frozen muscle were considered satisfactory for this purpose, because the specific activity of the enzyme was about six times higher than that in whole homogenates and about ten times higher than in supernatants of the homogenates (Table 1). The mean values obtained for each assay were comparable in preparations from fresh and frozen muscles.

Table 1. Activities of palmityl CoA synthetase, carnitine acetyltransferase and carnitine palmityltransferase in human muscle. The activities are expressed as nanomoles of product formed per minute per milligram of protein (± S.E.). Control muscles were from six normal volunteers (vastus lateralis) and from non-weak patients undergoing operations (pectoral and gastrocnemius). Number of controls is in brackets. The values for the patient refer to two biopsies (right and left vastus lateralis).

Enzyme	Preparation	Enzyme activities	
		Controls	Patient
Palmityl CoA synthetase	Homogenate	2.77 ± 0.17 [9] (2.4–3.9)	2.21
Carnitine acetyltransferase	1000*g* supernatant	20.9 ± 1.6 [6] (16.0–27.2)	12.0
Carnitine palmityltransferase			
Hydroxamate	Homogenate	2.3 ± 0.2 [14] (1.2–3.8)	0; 0; 0
	Mitochondria	13.6 ± 1.0 [13] (8.6–19.0)	0.98; 1.20; 2.66
Isotope exchange	1000*g* supernatant	0.72 [3]	0; 0.17
	Mitochondria	6.8 ± 1.21 [8] (3.6–12.5)	0.64; 1.07
"Forward" radioactive	Mitochondria	16.6 ± 2.4 [5] (11.3–23.7)	3.3

Table 2. Respiratory and phosphorylative studies on isolated muscle mitochondria. Respiration is expressed as microatoms of oxygen per hour per milligram of protein. The number of controls is shown in brackets. The ranges of values are shown in parentheses. ADP/O, ratio of adenosine diphosphate to oxygen; RCI, respiratory control index.

Subject	Respiration	ADP/O ratio	RCI
	Pyruvate + malate		
Controls [7]	9.1 (5.2–13.7)	3.4 (2.7–4.1)	4.8 (2.3–7.0)
Patient	9.5	3.4	7.7
	Succinate (+ rotenone)		
Controls [9]	12.2 (7.0–20.2)	2.1 (1.7–2.6)	3.8 (2.2–7.5)
Patient	15.5	2.3	2.8
	Palmitylcarnitine (+ malate)		
Controls [3]	3.7 (3.2–4.0)	3.6 (2.9–3.7)	6.8 (3.0–9.6)
Patient	2.3	2.6	1.7

No carnitine palmityltransferase activity was detected in the patient's muscle homogenate by the hydroxamate method. By the same method, the activity in the patient's mitochondrial fraction was about 12 percent of the mean of 13 controls (Table 1). By the "isotope exchange" method, carnitine palmityltransferase activity in both muscle extract and mitochondria was also 12 percent of the controls. Slightly higher activity (20 percent of the controls) was found in the patient's mitochondria by the "forward" radioactive assay method (Table 1). Nine determinations were performed in two muscle biopsies from the patient, on both fresh and frozen muscle preparations.

To investigate the functional consequence of this enzyme defect on the utilization of long-chain fatty acids in vitro, we isolated mitochondria from fresh muscle (*12*). Respiration and phosphorylation were monitored with an oxygen electrode (*12*), and $^{14}CO_2$ production from [^{14}C]palmitate was measured by the method of Lin *et al.* (*13*).

Respiratory and phosphorylative functions of the patient's mitochondria were normal with a number of substrates (Table 2). With palmitylcarnitine, maximum respiration was 38 percent lower than the mean control value, the respiratory control index was poor, but the ratio of adenosine diphosphate to oxygen was normal. Production of $^{14}CO_2$ from [^{14}C]palmitate was undetectable after 5, 10, 15, and 30 minutes of incubation. The mean palmitate oxidation for three preparations of normal mitochondria was 1.3 nmole of $^{14}CO_2$ per minute per milligram of protein. In the patient's family, his brother was affected, and the parents were asymptomatic, suggesting autosomal recessive inheritance. The lack of symptoms in any organ other than muscle suggests separate genetic control of this enzyme in different tissues, as is the case for phosphorylase and other enzymes.

There seem to be two palmityltransferases; one (transferase I or A), located on the outside of the mitochondrial barrier, catalyzes the formation of palmitylcarnitine, and the other (transferase II or B), located on the inside of the mitochondrial barrier, catalyzes the reverse reaction, with formation of palmityl-CoA from palmitylcarnitine. Evidence for this dual localization of the enzyme was provided in liver mitochondria (*11*) where transferase I was bound to the external surface and transferase II was bound to the internal surface of the inner membrane.

Bressler (*14*) predicted three possible alterations of the carnitine transferase system: (i) a defect of carnitine; (ii) a defect of carnitine palmityltransferase I; and (iii) a defect of carnitine palmityltransferase II. The first defect was described (*15*) in a woman with progressive weakness, lipid accumulation in muscle, but no cramps or myoglobinuria. Our data demonstrate a defect of the carnitine palmityltransferase system, but whether this is a defect of transferase I, transferase II, or both is not clear.

The studies of the utilization of palmitate as opposed to palmitylcarnitine by our patient's mitochondria suggest that his deficiency is primarily a deficiency of carnitine palmityltransferase I, with some functionally significant residual activity of the transferase II. Transport of acetyl-CoA and short-chain (up to four carbon atoms) acyl-CoA's depends on a different carnitine acyltransferase (*16*). The 40 percent decrease of acetylcarnitine transferase activity in this patient's muscle suggests some common genetic control of the two systems. On the other hand, the high content of carnitine in the patient's muscle may represent a secondary compensatory mechanism for the deficiency of palmityltransferase. The pathogenesis of myoglobinuria is obscure, but acute muscle necrosis often seems to be due to a failure of energy production (*17*). In the glycogen storage diseases due to lack of muscle phosphorylase or phosphofructokinase, strenuous exercise in essentially anaerobic conditions would result in a defect of adenosine triphosphate (ATP) production due to the block of glycolysis. A similar but speculative mechanism could be involved in the defect of long-chain fatty acyltransferase; while glycogen is the substrate preferentially used by skeletal muscle in short, strenuous exercise, fatty acids are preferentially utilized by muscle at rest or in sustained exercise of moderate intensity (*18*). If utilization of fatty acids is impaired, then ATP production may also be deficient, particularly in conditions of glycogen depletion by exercise or starvation.

If lipid metabolism is severely impaired, the question arises as to why there was no abnormal accumulation of lipid in the muscle of our patient, especially since excess lipid was found by Engel and Angelini (*15*) in their patient with decreased muscle carnitine. Also unanswered is the question of why the clinical picture of carnitine palmityltransferase deficiency was dominated by intermittent myoglobinuria without weakness, while progressive weakness without myoglobinuria was the main symptom of the patient with carnitine deficiency.

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10. Muscle samples (1 to 3 g), frozen and stored in liquid nitrogen, were thawed in nine volumes of ice-cold 0.15*M* KCl, 50 m*M* tris-HCl, *p*H 7.5, finely minced, and homogenized in a glass homogenizer with a Teflon pestle. The homogenate was centrifuged at 1,000*g* for 15 minutes, and the supernatant was passed through two layers of cheesecloth and centrifuged at 14,000*g* for 20 minutes. The mitochondrial pellet was rinsed gently with medium to remove the superficial fluffy layer, and resuspended in 0.5 to 1.0 ml of extraction medium. As judged by ultrastructural examination (E. Bonilla) these fractions appeared remarkably pure, and the mitochondria were well preserved.
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Ontogeny and Peripheralization of Thymic Lymphocytes

Abstract. *Reciprocal transplantation of undifferentiated thymic primordia between diploid and triploid chromosomally marked frog embryos has revealed that thymic lymphocytes are ontogenically derived from elements of the thymic primordium rather than from blood-borne stem cells that migrated into the developing organ. Virtually all the lymphocytes in the spleen, kidneys, and bone marrow of adult frogs are descendants of these original thymic stem lymphocytes.*

The embryonic derivation of thymic lymphocytes and of lymphocytes in the peripheral lymphoid tissues has been controversial (*1*). Studies of 12-day-old mouse thymic rudiments cultured in vitro (*2*) suggested that some epithelial cells of the rudiment itself transformed into lymphocytes. However, the argument that lymphocyte precursor cells had already migrated into the thymic epithelium from the blood islets of the yolk sac by 12 days of gestation (*3*) is consonant with the theory currently in vogue that during thymus differentiation, thymic lymphocytes are derived from invading hemocytoblast-like cells rather than from epithelial cells. This hypothesis has received substantial support from a variety of experimental protocols (*4*), most notably those in which vascular migration pathways of chromosomally labeled cells have been determined in 7- to 8-day-old chick embryos (*1, 5*).

We have reinvestigated the question of the thymic versus the extrathymic origin of lymphocytes in the developing thymus and peripheral lymphoid organs in an amphibian system. The embryo of the leopard frog, *Rana pipiens*, has provided us with a model in which we have been able to identify and chromosomally label the presumptive thymic tissue, remove it at a very early embryonic stage (even before it can be called a rudiment) from an embryo whose vascular system and blood islands are still undifferentiated, and transplant it orthotopically (either unilaterally or bilaterally) to an unlabeled recipient of the same age. We have been able to rear these chimeric embryos through metamorphosis and then quantitatively determine the relative percent of donor and host cells that populate in the differentiated transplant and the lymphoid organs of the host. Such procedures, which are necessary to resolve this question, are technically impossible with murine embryos.

The following experiments reveal that, at least in the frog, thymic lymphocytes arise in situ from components in the presumptive thymus area of very early embryos. After thymic differentiation, these lymphocytes leave the thymus and seed the peripheral lymphoid organs.

To verify the suspected location of the presumptive thymus tissue, we microsurgically extirpated the gill buds from avascular 72-hour (3-mm) tail bud

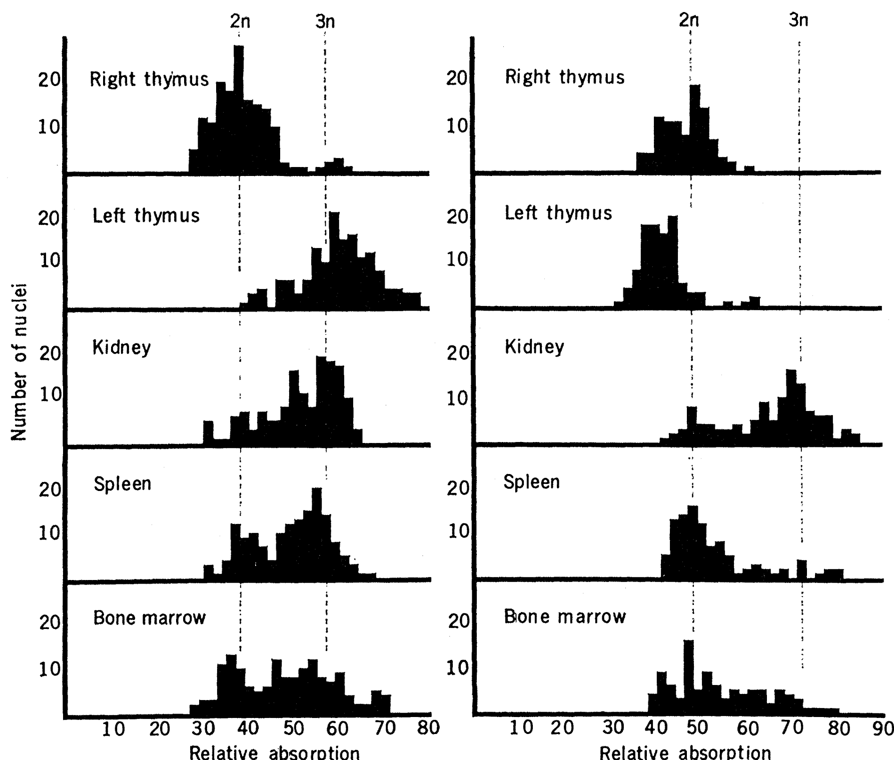

Fig. 1. Frequency distribution of absorption measurements of Feulgen-stained nuclei of tissues from two postmetamorphic triploid frog hosts. One recipient (left) had been grafted in embryonic life with one orthotopic diploid gill (thymic) primordium from the right side of a donor (unilateral exchange). Histograms at right are derived from a triploid host whose paired thymic primordia had been replaced in embryonic life with the left and right donor (diploid) gill buds (bilateral exchange). Dashed vertical lines indicate absorption maxima for each ploidy class (*12*).