in the lumbar CSF of man is derived largely from rostral sources and not from the spinal cord. The results are consistent with those of Curzon *et al.* (*8*), who found lower HVA concentrations in CSF of patients with CSF blocks in the cervical region as compared to controls. The low values for HVA in patients with CSF block also are consistent with animal studies showing no appreciable amounts of dopamine in the spinal cord (*4–6*).

The amount of MHPG in the CSF was similar in patients with obstructed CSF flow and in those without block. If brain sources of MHPG make a major contribution to the CSF levels, then one would expect lower amounts of this metabolite in patients with CSF block compared to those without block. This appears to be the first evidence that norepinephrine metabolism in the spinal cord contributes to MHPG levels in the lumbar CSF of man.

The fact that the MHPG values for the total patient group are significantly lower than normal may indicate that a portion of the spinal cord source for MHPG involves descending spinal tracts, which degenerate after transection. It is also possible that the low MHPG values reflect low levels of motor activity in these para- and quadraplegic patients. This would be consistent with the demonstration that experimentally induced increases in psychomotor activity produce higher concentrations of HVA, 5HIAA, and MHPG in CSF of depressed patients (*21*).

Values for 5HIAA were normal in patients with spinal cord transection and were the same whether CSF block was present or absent. Definitive interpretation of these data is difficult without knowledge of serotonin metabolism in the transected human spinal cord. If concentrations of serotonin and 5HIAA in the cord are reduced after transection in man as they are in animals (*5, 6, 10–13*), then the normal 5HIAA values found in the CSF of patients without CSF block would suggest a contribution from rostral sources. However, the limited data in patients with CSF block may indicate that the spinal cord below the level of the block contributes to 5HIAA concentrations in CSF. Although histofluorescence studies in animals detected no serotonin or norepinephrine after transection, the biochemical studies showing residual serotonin in the cord (12 to 25 percent of normal) led to the suggestion that interneurons, which do not degenerate after cord section, are a source of 5HT and 5HIAA (*5, 6, 10–13*). In animals with transected cords, serotonin precursors and antagonists respectively facilitate and inhibit monosynaptic reflexes, results that also provide evidence for a serotonin interneuron (*13, 22*).

In man, the amount of serotonin and 5HIAA remaining in the cord after transection may be greater than in the other species studied. Moreover, the relatively long age of the lesions in this patient population might allow for compensatory increases in serotonin function below the lesion. This, as well as variability in degree of CSF block, might explain the differences between our results and those of Curzon *et al.* (*8*), who found low values for 5HIAA in patients with CSF block.

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Carnitine Deficiency of Human Skeletal Muscle with Associated Lipid Storage Myopathy: A New Syndrome

Abstract. *In a rare myopathy muscle fibers contained myriad lipid-filled vacuoles. Homogenates of the patient's muscle oxidized fatty acids more slowly than normal (11 controls). Addition of carnitine increased the oxidation rate with the patient's muscle to the level attained by the controls with carnitine. In five separate muscle samples from the patient the mean carnitine level was less than 20 percent of that observed in 42 controls. Carnitine palmityl transferase and palmityl thiokinase levels in the patient's muscles were not depressed. The present case represents the first recognized instance of carnitine deficiency in human skeletal muscle.*

Carnitine (γ-trimethylamino-β-hydroxybutyrate) catalytically stimulates the oxidative catabolism of long-chain fatty acids by facilitating their transport from the cytoplasm to intramitochondrial sites where they can undergo beta oxidation (*1*). Carnitine deficiency in fetal bovine (*2*), neonatal rat (*3*), or diphtheritic guinea pig myocardium (*4*) and in heart and liver of choline-deficient rats (*5*) is associated with depressed long-chain fatty acid oxidation and excessive triglyceride formation. We here report the first recognized instance of carnitine deficiency in human skeletal muscle with associated lipid storage myopathy.

A 24-year-old woman has been

investigated at the Mayo Clinic since age 19. Clinical, histochemical, and ultrastructural observations in this patient have been reported (*6*). She had slight muscle weakness all her life which became progressively worse in her 19th year. Muscle biopsies revealed a vacuolar myopathy, and histochemical studies indicated that sudanophilic lipid droplets filled the abnormal spaces in the muscle fibers. Prednisone therapy (decreasing from 60 to 27.5 mg/day) improved the patient's weakness and improved, but did not remove, the histologic abnormalities. During July 1972 the prednisone dosage was reduced to a replacement level (7.5 mg/day).

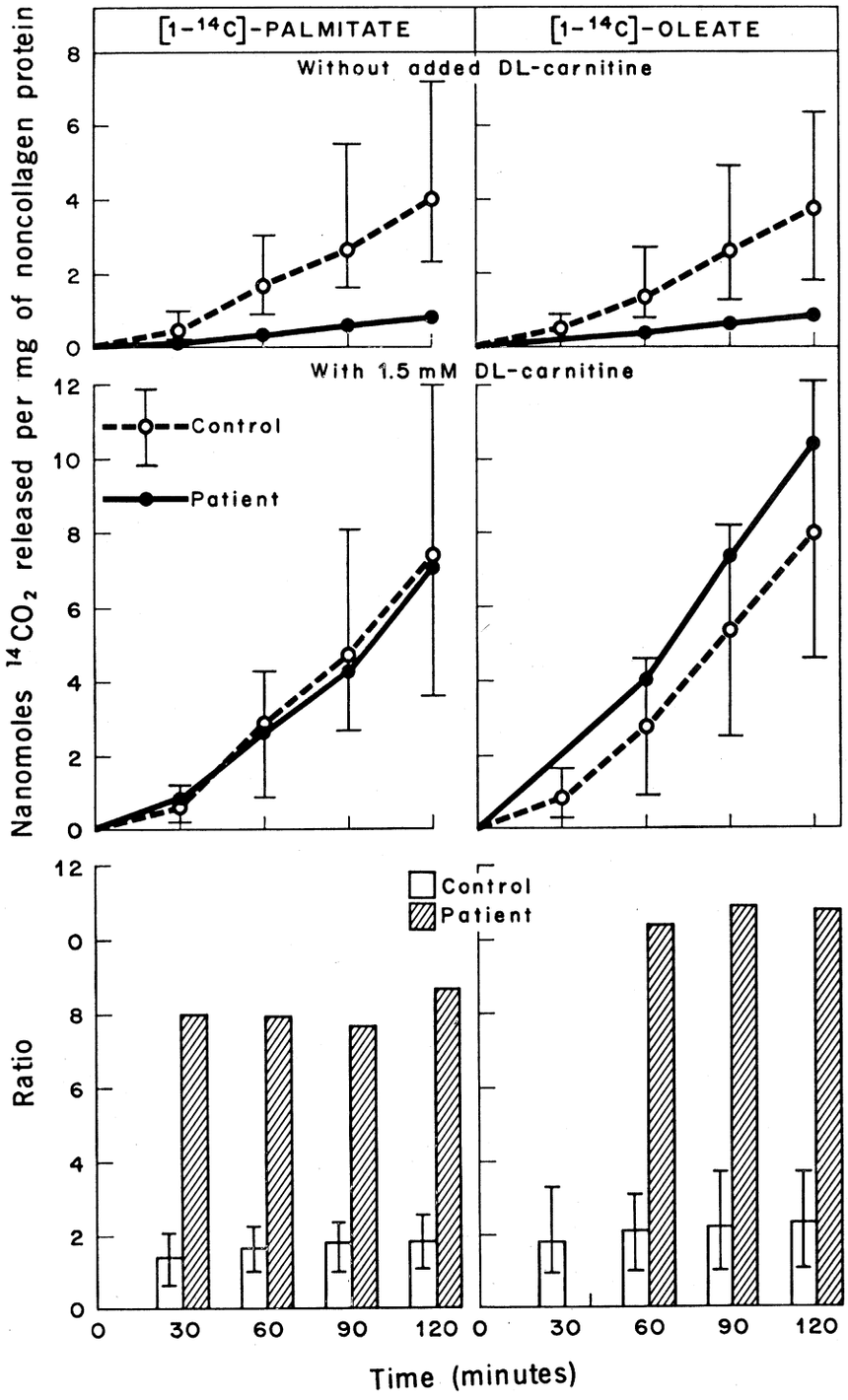

Fig. 1. Effects of carnitine on long-chain fatty acid oxidation by homogenates of patient's muscle and 11 control muscles. Labeled palmitate (left panels) and oleate (right panels) were used without (top panels) and with (middle panels) added DL-carnitine. In the upper four panels, plotted points and vertical lines for controls indicate mean and range of values. Observations with oleate as substrate were not available for patient at 30 minutes. In the lower two panels, columns indicate ratios of activities obtained with carnitine to activities obtained without carnitine. For controls the columns and vertical lines represent mean and range of ratios.

To investigate the possibility that the muscle lipid excess in the present case was a consequence of a defect in the oxidative catabolism of fatty acids, homogenates of the patient's rectus femoris muscle (biopsy date, 26 July 1972) were incubated with labeled substrates by a modification of the method employed by Lin *et al.* (*7*). Limb, pectoral, or abdominal muscles of 11 patients undergoing surgical procedures but not having muscular weakness served as controls. The noncollagen protein of the muscle homogenates was digested as described by Lilienthal *et al.*, and the protein concentration of the digests was determined by Lowry's method (*8*).

Figure 1 depicts the oxidation of two long-chain fatty acids, with and without added carnitine, by the patient's muscle and by 11 control muscles. Without carnitine the patient's muscle oxidized either palmitic or oleic acid at a slower rate than any of the controls. By contrast, when carnitine was added to the reaction mixture the patient's muscle oxidized both long-chain fatty acids at essentially the same rate as they were oxidized by controls with added carnitine. Among all the controls, at any time during incubation with substrate, the maximal observed increase of the oxidation rate by carnitine was 2.5-fold for palmitic and 3.7-fold for oleic acid. With the patient's muscle, the increase of oxidation rate by carnitine was 7.7- to 8.7-fold for palmitic acid and 10.4- to 10.8-fold for oleic acid. The oxidation of β-hydroxybutyrate, pyruvate, and succinate by the patient's muscle was in the range of control values.

The much greater stimulation by carnitine of long-chain fatty acid oxidation with the patient's muscle than with control muscles suggested that the endogenous carnitine content of patient and control muscles might be different. For this reason carnitine levels were determined according to the procedure of Marquis and Fritz (*9*) in biopsy specimens that had been preserved in liquid nitrogen. Five specimens of the patient's muscle (obtained between 1967 and 1972), limb, pectoral, or abdominal muscles of 18 patients not having muscular weakness, and limb or pectoral muscles of 24 patients affected by

various neuromuscular disorders (listed in Table 1) were assayed for carnitine. The mean carnitine level in the patient's muscle specimens was 16 percent of the nonweak control mean ($P < .001$), and 19 percent of the neuromuscular disease control mean ($P < .001$). The nonweak and neuromuscular disease control means did not differ significantly (Table 1).

Because in neonatal or fetal animal tissues low carnitine levels can be associated with decreased activities of long-chain fatty acid thiokinase and carnitine palmityl transferase (*2, 3*), we also measured these enzymes in three muscle biopsy specimens obtained from the patient in 1972 and in muscle samples from ten nonweak controls. Long-chain fatty acid thiokinase was determined with palmitate as substrate (i) by the hydroxamate method of Pande and Mead (*10*) and (ii) as described by van Tol and Hülsmann (*11*). Carnitine palmityltransferase was assayed by (i) the "isotope exchange reaction" and (ii) by the "forward reaction" as described by van Tol and Hülsmann (*11*). The levels of long-chain fatty acid thiokinase in the patient's muscles with the first method ranged from 212 to 221 nmole of hydroxamate formed per minute per gram (wet weight), and with the second method, from 222 to 270 nmole of palmitylcarnitine formed per minute per gram (wet weight). The corresponding values (mean ± S.E.) for ten control muscles were 129 ± 9.5 and 149 ± 11.4, respectively. The activity of carnitine palmityltransferase in the patient's muscles ranged from 79 to 106 and from 152 to 174 nmole of palmitylcarnitine formed per minute per gram (wet weight) by the first and second methods, respectively. The corresponding values (mean ± S.E.) for ten control muscles were 76 ± 7.2 and 79 ± 5.9. The increased enzyme activities in the patient might have been due to the decreased concentration in carnitine-deficient muscle of metabolites acting as allosteric inhibitors, or possibly the low muscle carnitine level can augment (or derepress) the synthesis of these enzymes.

Our findings are consistent with the assumption that the metabolic defect in muscle in the present case is a decreased transport of long-chain fatty acids into mitochondria due to carnitine deficiency. The enzymes subserving the activation of long-chain fatty acids with coenzyme A and their esterification with carnitine were either intact or overly active, while those enzymes subserving beta oxidation of acyl groups and the oxidation of citric acid cycle substrates were intact.

Table 1. Muscle carnitine levels. Biopsy dates (day, month, year) are in parentheses.

Source	Carnitine (nmole/mg of noncollagen protein)
Patient	
Right lateral vastus (7/7/67)	4.44
Left triceps (30/4/69)	1.15
Right lateral vastus (30/6/72)	1.31
Left femoral rectus (26/7/72)	2.97
Left lateral vastus (13/9/72)	1.38
Mean ± S.E.	2.25 ± 0.64
Nonweak controls	
Mean ± S.E.	13.92 ± 0.92
Range	7.96 to 22.86
N	18
*Neuromuscular controls**	
Mean ± S.E.	12.08 ± 0.78
Range	7.43 to 20.75
N	24

* The following were represented: Duchenne dystrophy (4); Becker dystrophy (1); limb-girdle dystrophy (2); myotonic dystrophy (2); dystrophy of ocular, pharyngeal, and limb muscles (1); polymyositis (5); motor neuron disease (2); neuromyopathy with malabsorption syndrome (1); thyrotoxic myopathy (1); myxedema myopathy (1); corticosteroid-induced myopathy (1); adult acid maltase deficiency (1); nemaline myopathy (1); and central core disease (1).

The reason for the muscle carnitine deficiency in the present case is not known. It could be caused by a defect in biosynthesis, or by excessive catabolism, or possibly by a failure of carnitine to gain access to the muscle fibers. It is not yet known whether carnitine is synthesized from precursors by each tissue de novo or synthesized in some tissues or organs (as liver) and then assimilated by others. If tissues other than muscle (heart or liver) of our patient were not deficient in carnitine, then this might implicate a defect in the transport mechanism of carnitine across the muscle fiber membrane. Additional studies are needed to investigate these possibilities.

The beneficial effect of prednisone in the present case was of special interest. As no correlation could be observed between the prednisone dosage and the muscle carnitine level in five different biopsies obtained over a 5-year period, the drug might have exerted its effect via a compensatory mechanism that can reduce muscle lipid levels. That glucocorticoids can decrease lipogenesis and increase fatty acid mobilization in tissues other than muscle has been previously observed (*12*).

Other instances of human myopathies associated with muscle lipid excess have been described (*13–15*). However, different biochemical defects can be associated with lipid accumulation in striated muscle. One patient, without weakness, suffered from an intermittent movement disorder and had an inherited defect in pyruvate decarboxylase (*13*). Two patients (identical twins), also without weakness, had cramping aches after exercise, fasting, or ingestion of a high-fat, low-carbohydrate diet; metabolic studies revealed a failure to form ketone bodies after fasting or while ingesting large amounts of long-chain fatty acids (*14*). Metabolic studies in our case (*16*) indicated a rise in serum and urinary ketone bodies under these conditions. These observations also suggest that the hepatic utilization of long-chain fatty acids may not be abnormal in the present case.

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Involvement of Adenosine 3′,5′-Monophosphate in the Activation of Tyrosine Hydroxylase Elicited by Drugs

Abstract. *Immediately after the injection of reserpine (16 micromoles per kilogram, intraperitoneally), aminophylline (200 micromoles per kilogram, intraperitoneally), and carbamylcholine (8.2 micromoles per kilogram, intraperitoneally), the concentration of adenosine 3′,5′-monophosphate in adrenal medulla of rats is increased severalfold. The three drugs also cause a delayed increase of medullary tyrosine hydroxylase activity. Our results are consistent with the view that an increase of medullary adenosine 3′,5′-monophosphate concentration is involved in the drug-induced increase of tyrosine hydroxylase activity in adrenal medulla. Experiments with tyramine (130 micromoles per kilogram, intraperitoneally) suggest that the increase of tyrosine hydroxylase activity and of adenosine 3′,5′-monophosphate concentrations is independent of an increase in adrenal catecholamine turnover rate.*

Tyrosine hydroxylase (TH) activity of adrenal gland is transsynaptically induced (*1*). Several studies have attempted to explore the molecular basis of this regulation; however, the mechanisms involved are still unclear. Kvetnansky *et al.* (*2*) reported that injections of dibutyryl adenosine 3′,5′-monophosphate (dibutyryl cyclic AMP) restored to normal the TH activity of adrenal glands of hypophysectomized rats. Recently, Waymire *et al.* (*3*) reported that the continuous presence of dibutyryl cyclic AMP in neuroblastoma tissue cultures increases the TH activity. Since sodium butyrate also elicits the increase in enzyme activity, one cannot infer that this activation is related to an increase of adenosine 3′,5′-monophosphate (cyclic AMP) without measuring the concentration of this nucleotide. Unfortunately, these measurements were not reported by Waymire *et al.* (*3*).

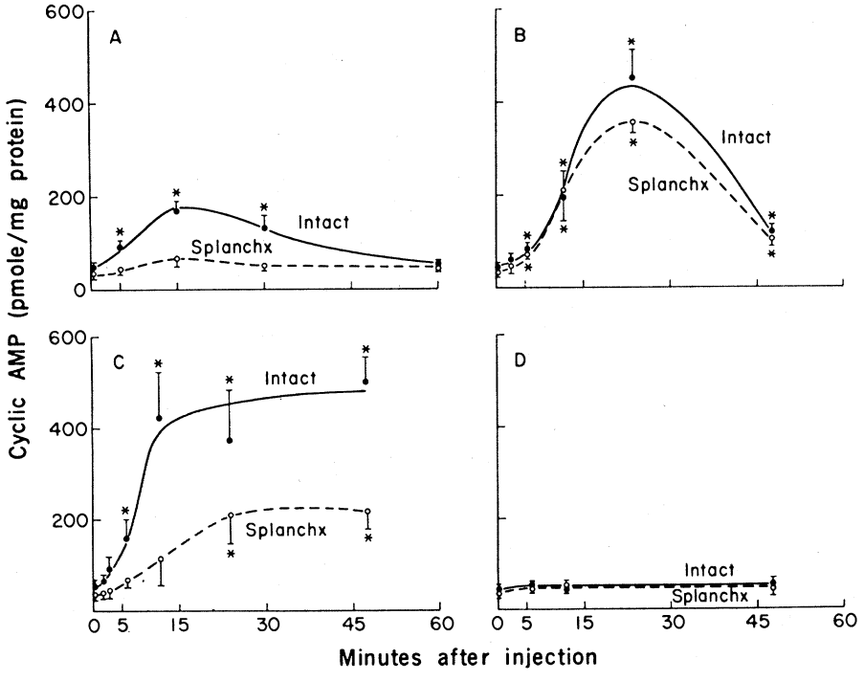

Fig. 1. Cyclic AMP concentration in intact and denervated (*Splanchx*) adrenal medulla of rats receiving intraperitoneally: (A) reserpine (16 μmole/kg), (B) carbamylcholine (8.2 μmole/kg), (C) aminophylline (200 μmole/kg), (D) tyramine (130 μmole/kg). Each point represents the mean values of at least five experiments. The concentrations of cyclic AMP at various times after injection of the solution used for dissolving reserpine or after saline were not different from those of rats not receiving saline. The vertical brackets indicate standard error. *, $P < .05$ compared with control animals.

The present experiments were carried out to verify whether the adenylyl cyclase system of adrenal medulla is involved in the increase of TH activity elicited by drugs. We measured the concentration of cyclic AMP and the activity of TH in intact and denervated adrenal gland of rats receiving various drugs. We studied reserpine because it increases adrenal TH activity (*4*), carbamylcholine because it stimulates adrenal catecholamine secretion (*5*), aminophylline because methylxanthines inhibit phosphodiesterase activity (*6*), and tyramine because it releases catecholamines from peripheral noradrenergic neurons (*7*). The results obtained support the working hypothesis that an increase in concentration of adrenal cyclic AMP is associated with a delayed enhancement of the TH activity.

Sprague-Dawley male rats (Zivic Miller Laboratories, Allison Park, Pa.) (about 180 g) were used. The left splanchnic nerve of these animals was severed 5 days before the experiment. Drugs were injected into monolaterally operated rats, and their effect on endogenous cyclic AMP and TH activity was measured in intact and denervated adrenal medulla. Adrenal medulla was dissected from cortex at about 4°C under a dissecting microscope. The accuracy of the dissection was ascertained by assaying the concentration of catecholamines (*8*) and corticosteroids (*9*). The medulla contained about 95 percent of the catecholamines while the cortex contained about 99 percent of the total corticosteroids present in the adrenal gland.

The concentration of cyclic AMP was measured by the method of Ebadi *et al.* (*10*) as successively modified in our laboratory (*11*). Tyrosine hydroxylase activity was measured by the method of Waymire *et al.* (*12*) with the use of carboxyl-labeled tyrosine (specific activity, 10 μc/μmole).

The intact adrenal medulla of rats receiving intraperitoneally reserpine (16 μmole/kg), carbamylcholine (8.2 μmole/kg), or aminophylline (200 μmole/kg) 6 minutes before being killed contains more cyclic AMP than