Effects of Carnitine on Fatty-Acid Oxidation by Muscle

The function of carnitine (β-hydroxy, γ-betaine butyric acid) in vertebrate organisms is not known. Carnitine is widely distributed among microorganisms, plants, vertebrates, and invertebrates (*1*), but the richest source is muscle. Fraenkel and his colleagues (*1*) have shown that the meal worm *Tenebrio molitor* requires a growth factor, initially named vitamin B_T, which has been identified as carnitine.

While examining the effects of muscle extracts on fatty-acid metabolism by liver, we had demonstrated that part of the action of muscle extracts could be simulated by the addition of carnitine, which resulted in augmented conversion of palmitate-1-C^{14} to $C^{14}O_2$ and ketones by both liver slices and homogenates (*2, 3*). Experiments have been performed in efforts to elucidate the site of carnitine action on the fatty-acid oxidase system. It has been established that carnitine effects cannot be duplicated by choline (*3*), and that carnitine cannot substitute for choline in preventing the development of fatty livers in rats maintained on a choline-deficient regimen; from these findings it was concluded that carnitine and choline influence different aspects of fatty-acid metabolism.

More recently, we have shown that the action of carnitine on the fatty-acid oxidase system is lost when the component enzymes from liver particulates are solubilized by treatment with deoxycholate or by homogenization of the particulates in distilled water in a Waring blender (*4*). Furthermore, addition of carnitine has no effect on the oxidation of palmityl-1-C^{14}-coenzyme A (CoA) (*4*); this suggests that carnitine action occurs prior to the formation of palmityl CoA in a particulate system.

Table 1. The effects of carnitine on palmitate oxidation by muscle particulates (*12*).

Albumin/palmitate molar ratio	*dl*-Carnitine hydrochloride added (μmole)	Palmitate-1-C^{14} converted to $C^{14}O_2$ per 100 mg (dry weight) of muscle per 30 min (mμmole)	
		Control	Carnitine
	Experiment No. 1		
0	0.762	7.97	14.7
0.14	0.762	24.0	34.8
1	0.762	5.31	44.3
2	0.762	2.40	27.1
	Experiment No. 2		
1	0.0254	7.79	9.30
1	0.254	7.79	25.2
1	2.54	7.79	61.5
1	10.2	7.79	54.8
1	19.0	7.79	20.5

Attempts to demonstrate an effect of carnitine on the long-chain fatty-acid activating enzyme described by Kornberg and Pricer (*5*) were negative, although a system was obtained which yielded proportionately increasing amounts of palmityl hydroxamate with the addition of rat-liver particulate enzyme preparations (*4*). The possibility remains that carnitine augmentation of long-chain fatty-acid oxidation is associated with facilitation of the transfer of palmitate to the active site of the fatty-acid oxidase particulate.

Results given in Table 1 indicate that the addition of carnitine enhances palmitate-1-C^{14} oxidation by washed rat-skeletal-muscle particulates. The effect is less than twofold at zero albumin concentrations, but the percentage increase induced by carnitine is augmented as bovine serum albumin is added. Higher amounts of albumin depress fatty-acid oxidation, presumably by firmer binding of the palmitate (*6, 7*). In the presence of carnitine, the albumin-induced inhibition of palmitate oxidation is considerably lessened. The effects of varying the carnitine concentration are also shown in Table 1, where it can be seen that carnitine action occurs over a wide range of concentrations, including the reported carnitine concentrations in rat skeletal muscle (*1*).

Since carnitine effects are evident in the absence of added albumin, it is unlikely that carnitine exerts its action on the metabolism of palmitate exclusively by uncoupling palmitate-albumin binding sites. However, it is not impossible that palmitate-protein sites may be influenced by the presence of the dipolar carnitine molecule, which has both lipophilic and hydrophilic groupings. Other instances are known in which cationic detergents, for example, can disrupt lipid from lipoprotein complexes (*8*). At any rate, the addition of carnitine is followed by augmented palmitate oxidation by muscle particulates, and the relative magnitude of the effect is remarkably increased at higher albumin-to-palmitate ratios.

In addition to the results of the present study, indicating the inhibition by high albumin-to-palmitate ratios of palmitate oxidation by muscle particulates, previous work has shown that the albumin-to-palmitate ratio influences the amount of fatty acid oxidized by isolated rat hemidiaphragm segments (*6*). When the albumin-to-palmitate ratio exceeded 0.14, approximately, palmitate oxidation was depressed (*6*). This is a somewhat curious phenomenon, since the albumin-to-palmitate ratio in mammalian plasma is around unity if one assumes an albumin concentration of 4 g/100 ml, an albumin molecular weight of 69,000, and a plasma nonesterified fatty-acid concentration of 0.5 to 0.6 m*M*. Serum albumin has the property of strongly binding palmitate (*7*), and recent evidence indicates that blood nonesterified fatty acids are transported as an albumin–fatty-acid complex (*9, 10*).

There is no doubt that muscle readily captures nonesterified long-chain fatty acids (*10*) and that it can oxidize them to CO_2 under conditions of both rest and activity (*6*). Since the available data suggest that palmitate oxidation by muscle *in vivo* would be inhibited by an albumin-to-palmitate ratio of unity, it is relevant to ask by what means serum fatty acids are transported from blood vessels through the interstitial space to the active sites for fatty-acid oxidation in muscle cells. The present data suggest the possibility that carnitine in muscle in some manner serves the function of facilitating fatty-acid transfer to the fatty-acid oxidase sites (*11*).

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11. This investigation was supported by research grants A-1465 and A-1682 from the National Institutes of Health, U.S. Public Health Service. The counting equipment used was purchased with the aid of a grant (A-133) from the Michigan Memorial Phoenix Project.
12. Fed male Holtzman rats of approximately 200 g were killed by decapitation. The hind leg musculature, freed of gross fat and fascia, was rapidly removed and placed in ice-cold 0.14*M* KCl, buffered to *p*H 7.4 with 0.015*M* phosphates. After several washes the weighed muscle was homogenized in 5 vol of this solution in an ice-jacketed Monel blender for 10 sec at a time at 60-sec intervals, for a total of 60 sec. The homogenate was rehomogenized in a Ten Broeck apparatus, strained through one layer of cheesecloth, and centrifuged at 4°C in a Servall angle centrifuge at approximately 4400*g* for 15 min. The residue was resuspended in the same volume of KCl-PO_4 medium and recentrifuged, as described above. The washed particulates were resuspended in one-fourth of the original volume of KCl-PO_4 solution and rehomogenized in a Ten Broeck tissue-grinding apparatus; after this 1.0-ml aliquots were transferred to Warburg-type vessels to give a total final fluid volume of 2.5 ml, containing 280 μmole of KCl; 6.5 μmole of $MgCl_2$; 30 μmole of KH_2PO_4 and K_2HPO_4, adjusted to *p*H 7.4; 5 μmole of adenosine triphosphate as the sodium salt (Pabst); 2.9 μmole of diphosphopyridine nucleotide (Sigma); 0.051 μmole of cytochrome *c* (Sigma); 0.225 μmole of CoA (Pabst); 0.285 μmole of K-palmitate-1-C^{14}, contain-

ing 0.5 μc of C^{14} (Nuclear) and crystallized bovine serum albumin as indicated (Armour). The *dl*-carnitine used was a gift from International Chemicals, Inc., Chicago, and was supplied as the hydrochloride. All flasks were incubated at 37°C for 30 min in air, after which 0.2 ml of 50-percent citric acid was added from a side arm to insure complete liberation of $C^{14}O_2$ in the medium. The $C^{14}O_2$ was trapped by alkali having filter paper immersed in the center well. The plating as $BaCO_3$ and the calculations employed were the same as those previously reported (*6*). The dry weight of muscle aliquots used in each flask was approximately 25 mg. The experiments reported are representative of six experiments performed in a similar fashion.

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Acrolein for the Control of Water Weeds and Disease-Carrying Water Snails

Abstract. Injection of the biocide acrolein into irrigation canals killed submersed weeds, thereby reducing flow resistance and increasing capacity. In a 20-mile canal, 150 gallons raised throughput from 311 to 552 ft³/sec. Acrolein also effectively eradicated aquatic snails; it promises to become a useful tool in the battle against *Schistosoma* blood flukes.

Irrigation is a blessing, for it turns deserts into productive lands, but there are certain drawbacks which must be overcome before the full benefits of irrigation can be realized. First, there is the menace of aquatic weeds, which, when left uncontrolled, soon render an irrigation system inoperative. Then, there is a menace to health, as several vectors of human and animal diseases are aquatic in habit. Various new irrigation systems in the tropics, for instance, introduce aquatic snails which are alternate hosts for a trematode worm (the blood fluke) which causes schistosomiasis (*1*).

Although ditch-bank weeds are difficult to control, submersed aquatic weeds constitute an even more difficult problem. It is the submersed weeds which cause the most trouble from the standpoint of reduced water flow in irrigation ditches (*2*). In the western United States alone farmers spend millions of dollars every year for the control of these weeds —commonly called "moss"—in canals and drains (*3*). Various mechanical methods—such as draining and drying, hand-cleaning, chaining, and dredging—for the control of submersed aquatic weeds in irrigation channels have been used extensively. In many situations, however, these methods have proved relatively inefficient or cumbersome, time-consuming, and expensive (*4*). Among chemical methods, use of copper sulfate was found to be effective on algae, but it was not generally effective on plants that were rooted in the bottom of the channel (*4*). Aromatic solvents such as crude xylenes gave effective control of submersed aquatic weeds but could be used economically only in smaller channels (up to 50 to 70 ft³ of water flow per second). Large amounts of solvent and emulsifier are required, and even when as much as 10 gal of solvent per cubic foot of water flow per second is applied in the channel, such an application is effective only for distances up to 5 mi without "booster" applications (*4*).

We have now developed a method, involving the use of a technical product the active ingredient of which is acrolein (CH_2:CH · CHO), which will control submersed aquatic weeds economically even in large canals (with water flow of 300 ft³/sec and over). Submersed weeds have been controlled as far as 15 to 20 mi below the point of application through the use of only 1 to 1.5 gal of acrolein per cubic foot of water flow per second over a period of 30 to 45 min. In less than 1 week after treatment the water-carrying capacity of such a large canal, 60 ft wide, nearly doubled, and the beneficial effect lasted for as long as 8 weeks before retreatment became necessary. Figure 1 shows the results of the water-weed control by acrolein in a large canal in Kern County, California (*5*). The major weed was the pond weed, *Potamogeton crispus*, but the chemical controlled all other submersed vegetation as well. After treatment the dead vegetation disintegrated and hence did not clog gates, weirs, and pumps, as happens after chaining (dragging). When deposited on the land, such masses of organic matter could be beneficial. Treated water, when used for irrigation, did not harm crops. Further studies, on possible acrolein residues in crops and on the toxicity of treated water with respect to farm animals, are being made.

Acrolein is a potent irritant and lachrymator, but in the hands of a skilled operator with proper application equipment it can be applied safely and without irritation or discomfort.

Acrolein readily forms a true solution in water and travels down the canal as a chemical wave. The location of the acrolein wave in the canal can be detected easily with a drop of potassium permanganate solution in a test tube. There is no loss of the chemical due to breaking of emulsions, as there is with aromatic solvents.

Acrolein has been found highly effective against water snails in the canals treated. Against adults of the pond snail *Lymnaea bulimoides,* and also against the local planorboid snail, *Helisoma* (Planorbis) *trivolvis,* it was found to be twice as effective, in laboratory tests in ½-gal jars, as sodium pentachlorophenate. The latter is commonly used for the control of bilharziasis (*6*). Against snail egg masses it was found to be more effective than copper sulfate, another agent commonly used as an aquatic molluscicide.

Acrolein is a potent sulfhydryl reagent and has been observed to destroy isolated enzyme systems. For instance, against urease it is nearly four times as toxic on a molar basis as ethyl maleimide, a common —SH reagent. Turgor in cells of the leaves of the water plant *Elodea densa* was maintained for a few hours after a dip in 1000 parts of acrolein per million, even though microscopic examination showed the cell interior to be destroyed. This observation leads to the conclusion that acrolein destroys —SH enzymes in the cytoplasm of the cell, unlike aromatic solvents, which act primarily on the plasma membrane.

Dosages required to kill *Elodea* leaf cells under laboratory conditions are small: 0.5 parts per million (ppm) for

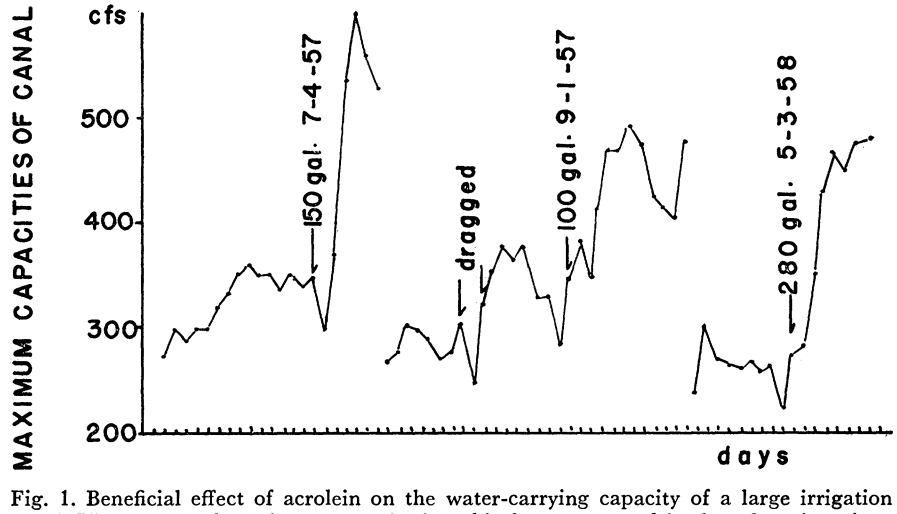

Fig. 1. Beneficial effect of acrolein on the water-carrying capacity of a large irrigation canal. The computed maximum capacity in cubic feet per second is plotted against time. The horizontal units are single days. The three trials reported refer to the same canal. The amount of acrolein and the date of treatment are indicated on each graph. The increase in flow is due to destruction of submersed water weeds by the chemical.