The Content of Citrate in Resting Muscles from Vertebrates and Invertebrates

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Summary. There is a marked difference between the content of citrate of anaerobic and aerobic muscles. The variation of the citrate content in insect flight muscles is very small.

Muscle tissue is characterized by its ability to perform mechanical work. The rate at which this work is done varies considerably according to the type of muscle and the animal from which it is derived. The mechanism of regulation of energy production involves citrate among other important regulators (e.g. ATP, ADP, AMP)[1]. Citrate inhibits both PFK[2-4] (phosphofructokinase E.C. 2.7.1.1) and citrate synthase[5,6] (E.C. 4.1.3.7).

Owing to the importance of these enzymes in metabolic regulation and to the multiplicity of metabolic roles played by citrate, it is somewhat surprizing that little information is available concerning the content of citrate in muscle tissues. A recent survey of the literature indicates that measurements of citrate in muscle in vivo are available only for muscle from the rat[7].

In this paper the content of citrate in a number of muscles from different animals under resting conditions is reported.

Citrate content of resting muscles

Animal	Muscle	Citrate content (μmol/g frozen tissue)
Mollusca		
Snail (*Helix pomatia*)	Foot	0.115 ± 0.037 (6)
Scallop (*Pecten maximus*)	Snap	0.036 ± 0.008 (6)
Sea mussel (*Mytillus edulis*)	Posterior adductor	0.040 ± 0.006 (6)
Crustacea		
Lobster (*Homarus vulgaris*)	Abdominal	0.086 ± 0.033 (5)
Insecta		
Locust (*Schistocerca gregaria*)	Flight	1.063 ± 0.066 (6)
Cockroach (*Periplaneta americana*)	Flight	0.639 ± 0.121 (6)
Cockroach (*Blaberus discoidalis*)	Flight	0.771 ± 0.078 (6)
Cockchafer (*Melolontha melolontha*)	Flight	1.342 ± 0.087 (5)
Water bug (*Lethocerus cordofanus*)	Flight	0.433 ± 0.122 (6)
Rosechafer (*Pachnoda ephippiata*)	Flight	2.084 ± 0.257 (6)
Dung-beetle (*Heliocopris* sp.)	Flight	1.461 ± 0.104 (6)
Honey-bee (*Apis melifera*)	Flight	1.397 ± 0.170 (6)
Blowfly (*Calliphora vicinia*)	Flight	1.514 ± 0.073 (5)
Pisces		
Dogfish (*Scylliorhinus canicula*)	White	0.182 ± 0.085 (5)
Amphibia		
Frog (*Rana temporaria*)	Gastrocnemius	0.079 ± 0.023 (5)
Reptila		
Green Lizard (*Lacerta viridis*)	Tail	0.042 ± 0.008 (5)
Aves		
Pigeon (*Columba livia*)	Pectoral	0.266 ± 0.050 (5)
Starling (*Sturnus vulgaris*)	Pectoral	0.255 ± 0.095 (4)
Domestic fowl (*Gallus gallus*)	Pectoral	0.058 ± 0.008 (5)
Mammalia		
Laboratory rat	Thigh	0.140 ± 0.017 6()
Laboratory mouse	Thigh	0.310 ± 0.067 (5)

Muscles were extracted and citrate measured as described in the Materials and Methods section. The results are reported as means ± SEM. with the number of individual muscles investigated given in parentheses.

Materials and methods. All chemicals and citrate lyase were obtained from Boehringer Corp. (London) Ltd., London W5 2TZ, U.K., except for the following: disodium EDTA and all inorganic reagents were obtained from BDH Chemicals Ltd., Poole, Dorset, BH12 4NN, U.K., and were of the highest purity available. Nembutal was obtained from Abbot Laboratories Ltd., Arge-Vet Division, Queenborough, Kent, U.K. Sandoz-MS22 was obtained from Thompson & Joseph Ltd., Castle House, Castle Meadow, Norwich, NOR 4ID, U.K.

Animals were obtained from sources given[8] with the addition of lizards which were obtained from Gerrard and Haig Ltd., East Preston, Sussex, U.K. All animals used were mature and were allowed free access to food and water before death.

Freeze-clamping of muscle. All muscle tissues used in the measurements of content of citrate were frozen rapidly with the aid of aluminium tongs cooled in liquid nitrogen. The method of removing and freeze-clamping the muscle depended upon the animal and the muscle under investigation. In each case, the method that was finally adopted represented a compromise between rapidity of freezing, selection of a specific muscle and freezing the muscle under resting conditions (see also Results section). For the snail, a piece of the foot was rapidly dissected and immediately freeze-clamped. For the other molluscs, the shell was opened by cutting through the muscle with a scalpel and a piece of muscle was rapidly dissected and freeze-clamped. From opening the shell to freezing the muscle, the procedure was completed in 5–10 sec. For the lobster, the animal was 'anaesthetized' by placing it in ice for 20 min, then the tail was cut off and a piece of abdominal muscle was cut away and freeze-clamped within 15 sec from removal of the tail. For the insects, the whole animal was freeze-clamped while at rest (i.e. non-flying). This procedure resulted in much of the flight muscle being squeezed out of the thorax,

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so that the muscle could be easily separated from cuticle and other tissues. In some insects, the muscle was dissected away from the cuticle at liquid nitrogen temperatures. For frogs, the animals were anaesthetised with Sandoz-MS222 (0.1 g/l water) and the skin was removed from the hind limb, the gastrocnemius muscle was separated from the other muscles and freeze-clamped: the dissection was complete within 30 sec. For dogfish, the animals were anaesthetized by i.v. (caudal vein) of Nembutal (0.1 ml/kg), a piece of white muscle was rapidly dissected and freeze-clamped. The dissection was completed within 30 sec. For the birds, the animals were anaesthetized by an i.p. injection of Nembutal, feathers and skin were rapidly removed from above the pectoral muscles and a piece of pectoral muscle was dissected and immediately freeze-clamped. From the initial incision to the freeze-clamping of the muscle, the procedure was completed within 5 sec. For rats and mice, the animals were anaesthetised with ether, the skin was dissected from the hind limb and the exposed muscle was freeze-clamped in situ. The muscle was dissected away from the bone at liquid nitrogen temperatures. The frozen muscle was powdered in a percussion mortar at −70 °C, and the powdered muscle was extracted by adding 4–5 volumes of frozen $HClO_4$ (6% w/v). The extraction took place in a mortar and continual mixing with the pestle thawed the mixture of $HClO_4$ and frozen muscle powder. The precipitated protein was removed by centrifugation and the extract was neutralized with 3 *M*-$KHCO_3$. Citrate was measured in the neutralized extract enzymatically[9].

Results and discussion. For 21 species of animals from several phyla, the contents of citrate show considerable variation (Table). The contents of citrate range from 0.036 to 2.084 μmol/g fresh weight (snap muscle of scallop and the flight muscle of rosechafer).

The mean values of the citrate content in the insects investigated is 1.07 μmol/g fresh weight, whereas the mean value of the rest of the muscles investigated is 0.134 μmol/g fresh weight, i.e. about 8-fold lower.

The variation of the content of citrate in the flight muscle of insects is about 5-fold, and if the water bug and the cockroach are excluded from consideration, the variation is only 2-fold.

The variation in the rest of the muscles examined is about 9-fold.

The results also indicate that there is a clear difference in the citrate content between the aerobic and anaerobic muscles, so the content of citrate of the aerobic muscles is much higher than that of anaerobic muscles. However, flight muscle PFK is not sensitive to citrate[10,11], therefore the importance of high citrate content of the insects' flight muscles is not clear.

It must be stressed that the measurements reported here were made on whole muscle preparations. Therefore there is no indication of the citrate content in the various cell compartments and consequently available to the different enzymes.

Unfortunately, at the present time satisfactory methods for measurement of intermediates within different cell compartments are not available and any interpretation that involves the use of a precise concentration of a metabolic intermediate must be made with caution.

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ATP Reception by the Tsetse Fly, *Glossina morsitans* West.

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Summary. Electrophysiological studies of the labellar sensillae of *Glossina morsitans* show that one cell in each LR7 sensillum responds to ATP at concentrations from 10^{-3} to 10^{-5} *M*.

Adenosine triphosphate (ATP) is an important feeding stimulant for mosquitoes[2,3], a flea[4], a tick[5] *Rhodnius prolixus*[6] and the tsetse fly[7]. This implies that these animals possess a chemoreceptor sensitive to ATP. Physiological evidence for such a receptor in *Glossina austeni* was sought by RICE et al.[8], and they conclude that a cell sensitive to ATP is housed in one of the labellar sensilla. No positive identification of the sensillum could be made however because they recorded from a nerve containing the axons of many receptors while stimulating the labellar lobes. Here I present evidence for an ATP-sensitive cell in the largest of the labellar sensilla, called the LR7 sensilla[8].

Preparation and electrophysiological methods. When a fly is not probing the LR7 sensilla are protected by the labellar lobes, and are not visible externally. To expose them for recording, a small gauge wire is tightened around the bulbous, proximal part of the haustellum of a CO_2 anesthetized fly. This part of the haustellum contains the retractor muscles of the labella which, on contraction, cause the labella to evert exposing the armature (prestomal teeth and rasping surfaces) and the LR7 sensilla[9]. The tightened wire probably causes these muscles to contract and, since the wire is left in place, the labella remain everted. In this manner 1 to 4 of the eight LR7 sensilla were made accessible.

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