Energy metabolism of swimming trout (Salmo gairdneri)

Oxidation rates of palmitate, glucose, lactate, alanine, leucine and glutamate

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Summary. The oxidation of 1-14C palmitate, 2-14C glucose, 1-14C lactate, 1-14C alanine, 1-14C leucine and 1-14C glutamate, injected via a cannula into the dorsal aorta, was determined in trout, either at rest, or during swimming at 80% of the maximum sustained speed. The oxygen consumption and the excretion rates of 14CO2 as well as CO2 were measured.

While the oxygen consumption of swimming trout was about twice as high as of resting trout, the oxidation rates of the injected tracers increased by up to 9 times. Despite the increased importance of blood borne substrates, the estimated contribution to total CO2 production is about 6% for the resting and 17% for the active trout. The majority of the oxidisable substrates must therefore be endogenous.

The mobilization and oxidation rates of lactate, palmitate and leucine were particularly increased during swimming. During rest, palmitate and leucine oxidation rates are low. While oxidation rates of alanine and glutamate are intermediate, those of glucose were found to be extremely low, both at rest and during swimming. The measured RQ values for resting and swimming trout were 0.91 and 0.96 respectively, indicating that protein is the major fuel, since glucose oxidation seems of minor importance.

Introduction

With respect to the use of energy sources by fish for sustained swimming, there is little information available (Bilinski 1974; Driedzic and Hochachka 1978). It has been observed that glycogen is mainly used during burst swimming, while during sustained swimming fat and protein are used as energy sources. Use of glycogen during sustained swimming appears to depend not only on swimming speed but also on the species. For example, glycogen depletion was found in coalfish (Johnston and Goldspink 1973) and mackerel red muscle (Pritchard et al. 1971) during sustained slow swimming, but not in salmon (Connor et al. 1964), nor in rainbow trout (Black et al. 1962). Sustained swimming resulted in depletion of lipid stores in mackerel (Pritchard et al. 1971) and in coho salmon (Krueger et al. 1968). Krueger et al. measured the effect of sustained swimming on protein and lipid depletion in coho salmon. They concluded that at high speed 16% of the caloric losses came from lipid. At lower speed, 45% came from protein. So it is obvious that protein may be an important energy source for the swimming trout. Similar results were found with resting animals. In feeding experiments with rainbow trout, Atherton and Aitken (1970) observed that feed protein can be oxidized to a great extent. Carbohydrates are metabolized poorly by rainbow trout (Lin et al. 1978) and given in the feed above 19% it is even found to be lethal (see Atherton and Aitken). Also with carp it is found that protein is preferred to carbohydrates as an energy source (Nagai and Ikeda 1971, 1972). The use of protein by fish in preference to fat and carbohydrates as an energy source is different from the extensively studied mammalian situation. So a further study on the use of energy sources seems necessary to understand exercise metabolism in fish. In this study we have tried to analyse the contribution of fat, protein and carbohydrates to the total energy production of resting trout and trout swimming at 80% of the maximal sustained speed.
Materials and methods

Respirometry. Experiments were carried out in a 3.71 m tunnel respirometer (as described by Brett 1964) operating at 10 °C. The respirometer was flushed with local tapwater. During the experiments the respirometer was closed and pure oxygen was injected to replace that used by the swimming fish, which permitted prolonged closure of the system without causing hypercapnia. Oxygen was injected, using a 50 ml calibrated glass Yare syringe kept at 10 °C and atmospheric pressure. The injected O₂ dissolved quickly in the water and was completely mixed within 15 min. Oxygen was injected only once after about 1h/2 h when the P₅₀ was at about 100 Torr.

The oxygen uptake was determined from the decline in oxygen content of the water and the amount of oxygen injected into the system. Because of the low buffering capacity of the tapwater, the pH had to be readjusted continuously to prevent hypercapnia. This was done by a pH-stat device consisting of a Radiometer PHM65 plus electrode and a small electrical comparator controlling a Harvard linear displacement pump, which injected 0.250 N NaOH (100% pure, CO₂ free) at rates between 0.5 and 1.5 ml/min. The pH was kept at 7.8 with a maximal variation of 0.05 pH units. The amount of injected NaOH was continuously recorded, from which the acid excretion during the experiment could be calculated.

The total CO₂ content of the water was determined by a modification of the method described by Cameron (1971) with a Radiometer PHM71 acid-base analyser and associated CO₂ electrode. The cuvette (2.5 ml) operating at about 40 °C was filled with water containing 0.3 to 1.0 mM NaHCO₃. The sample was acidified with 50 μl 2N HCl and TCO₂ was measured after 120 sec. The meter was calibrated with 0.6 and 0.3 mM NaHCO₃ every day, while during the experiments the 0.6 mM setting was checked every hour, since the value tends to shift about 2% per hour. This method gave a linear calibration line with a reproducibility of about 2% between 0.3 and 1.0 mM NaHCO₃. Prior to each experiment, NaHCO₃ was added to ensure a TCO₂ level of about 0.3 mM.

In all experiments the dorsal aorta of the fish was cannulated (Smith 1978) and an indwelling polyethylene cannula (PE50, Becton-Dickinson) was used for injection of radio tracers as well as for blood sampling. Cannulated fish were allowed two days recovery from the operation before being placed in the respirometer. The critical velocity (U crit ) of each fish was determined after an overnight rest in the respirometer. The water velocity was increased every hour by 0.5 bodylengths (BL) per second until the desired speed was reached. Then the respirometer was closed, NaHCO₃ was added and pH-stat activated. After about 30 min the tracer was injected. Samples for ¹⁴C measurements were taken initially every 5 min, later on every 15 min. Samples for P₅₀ and TCO₂ measurements were taken every 15 min. Samples for P₅₀ and TCO₂ were taken with a 10 ml B&D syringe and for ¹⁴C-activity with a 30 ml calibrated yale syringe via a 3-way valve.

Mathematical analysis. ¹⁴CO₂ evolution curves were analysed according to methods described by Hetenyi and Norwich (1974), and Lavau and Susini (1975). Data were fitted to a 3-compartment model by a computer iteration programme.

Results

Respirometry

Oxygen uptake and CO₂ and hydrogen ion production rates of 12 experiments are presented in Table 1. Four different fish were used and each fish was used for at least one complete experiment, i.e. with resting and swimming fish. During all experiments M O₂ and M CO₂ remained fairly constant; the acid production (M H⁺) though, was always higher at the beginning than at the end of each experiment. When we take the M H⁺ values over the last 60 min of all experiments, they are almost equal to the M CO₂ values, which is expressed by a M H⁺/M CO₂ ratio of 0.996 ± 0.185. When this value equals 'one', it indicates that CO₂ is the sole acid excreted by the fish into the medium. So, it appears that in all experiments trout excreted initially excess acid. The respiratory quotients (M CO₂/ M O₂) ranged between 0.84 and 1.03, but stayed rather close to 0.9.

CO₂-flux

Mixing and excretion of injected ¹⁴C-NaHCO₃ was measured in two experiments, with respectively, resting and swimming (80% U crit ) trout. In