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The energetic cost of protein synthesis in isolated hepatocytes of rainbow trout *(Oncorhynchus mykiss)*

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Summary. To establish the energetic cost of protein synthesis, isolated trout hepatocytes were used to measure protein synthesis and respiration simultaneously at a variety of temperatures. The presence of bovine serum albumin was essential for the viability of isolated hepatocytes during isolation, but, in order to measure protein synthesis rates, oxygen consumption rates and RNA-to-protein ratios, BSA had to be washed from the cells. Isolated hepatocytes were found to be capable of protein synthesis and oxygen consumption at constant rates over a wide range of oxygen tension. Cycloheximide was used to inhibit protein synthesis. Isolated hepatocytes used on average $79.7 \pm 9.5\%$ of their total oxygen consumption on cycloheximide-sensitive protein synthesis and $2.8 \pm 2.8\%$ on maintaining ouabain-sensitive Na^+/K^+-ATPase activity. The energetic cost of protein synthesis in terms of moles of adenosine triphosphate per gram of protein synthesis decreased with increasing rates of protein synthesis at higher temperatures. It is suggested that the energetic cost consists of a fixed (independent of synthesis rate) and a variable component (dependent on synthesis rate).

Key words: Energetic cost – Protein synthesis – Cycloheximide – Temperature – Teleost fish – *Oncorhynchus mykiss*

Introduction

After a meal the tissues of rainbow trout increase their rates of protein synthesis although there are large differences in the time-course of response between each individual tissue (McMillan and Houlihan 1989). It has been suggested that the post-prandial increases in O_2 consumption following feeding are a reflection of the energy cost of this surge in protein synthesis, with the total animal's O_2 consumption representing the sum of the increases in O_2 demand of the individual tissues (Lyndon et al. 1989; Houlihan 1991). In order to test this hypothesis values for the energy cost of protein synthesis are needed. Estimates of this cost are mostly based on theoretical calculations (Waterlow et al. 1978; Hawkins et al. 1989) or on correlations between whole animal O_2 consumption and protein synthesis (Reeds et al. 1985; Aoyagi et al. 1988; Hawkins et al. 1989). There are wide differences in the estimates of protein synthesis costs resulting from these "correlative" approaches (Aoyagi et al. 1988). Theoretical calculation of the cost of protein synthesis in fish range from 50 to 100 mmol ATP per g protein synthesis, depending on the assumptions made in the calculations (Jobling 1985). There is, however, a great difference between the two methods of cost estimates. Waterlow and Millward (1989) estimated a five-fold difference between the theoretical and "correlative" cost in mammals.

This paper addresses the question of the relationship between O_2 consumption and protein synthesis in a single fish tissue, the liver. The liver has been found to have one of the highest in vivo fractional rates of protein synthesis in fish (Fauconneau 1985; Simon 1987; McMillan and Houlihan 1989), and isolated fish hepatocytes have been used for a number of metabolic studies (e.g. Campbell et al. 1983; Baksi and Frazier 1990). A number of studies have measured the incorporation of radiolabelled amino acids into proteins by isolated fish hepatocytes (e.g. Saez et al. 1982; Haschemeyer and Mathews 1983; Plisetskaya et al. 1984; Bhattacharya et al. 1985; Koban 1986). In this study isolated trout hepatocytes have been used to measure O_2 consumption and protein synthesis simultaneously, the latter determined using an in vitro adaptation of the incorporation of [^{3}H]PHE into protein with the flooding-dose technique (Garlick et al. 1980). O_2 consumption and protein synthesis have been measured at a variety of temperatures

Abbreviations: BSA bovine serum albumin; dpm disintegrations per min; k_s fractional rate of protein synthesis; HEPES N-2-hydroxyethylpiperazine-N′-2-ethane sulphonic acid; PHE phenylalanine; PO_2 oxygen tension; PCA perchloric acid

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and the sensitivity of protein synthesis to the PO_2 of the incubation medium determined. Cycloheximide, a specific inhibitor of protein synthesis which blocks the peptidyl transferase reaction on ribosomes in eucaryotes (Alberts et al. 1983), was used to determine the extent of protein synthesis-related O_2 consumption. Cycloheximide has been used to inhibit protein synthesis in whole fish (Nichols and Fleming 1990; Brown and Cameron 1991), fish hepatocytes (Kent and Prosser 1980; Saez et al. 1982) and in a number of mammalian tissues (e.g. Emmerich et al. 1976; Siehl et al. 1985; Goodman 1987). Ouabain has been used to examine the energetics of maintaining Na^+/K^+-ATPase activity in trout hepatocytes (Bianchini et al. 1988).

Materials and methods

Animals. Rainbow trout *(Oncorhynchus mykiss)* of both sexes weighing between 300 and 400 g were obtained from a local farm. Fish were kept in outside tanks supplied with dechlorinated tap water at a temperature of 10 ± 2 °C and fed commercial trout pellets (Ewos, UK) twice a day ad libitum. Hepatocytes were prepared from fish 24 h after their last meal.

Chemicals. Collagenase type IV, cycloheximide crystalline; BSA, fraction V; ouabain octahydrate (Strophanthin G); and L15 Leibovitz Cell Culture Medium were obtained from Sigma (UK); L-[2,6-^{3}H]PHE at 37 MBq · ml^{-1} was purchased from Amersham International (Amersham, Buchs., UK).

Buffers and media. The perfusion buffers for the isolation of viable trout hepatocytes were adapted from Moon et al. (1985). pH was adjusted to 7.4. All buffers and incubation media were kept on ice and continuously equilibrated with 99.5% O_2/0.5% CO_2 using a gas mixing pump (Wösthoff, FRG). Perfusion buffer B (first washing perfusion) and perfusion buffer C (digestion) were as described by Moon et al. (1985). Washing buffer I consisted of perfusion buffer C without collagenase and $CaCl_2$; washing buffer II consisted of washing buffer I without BSA; incubation medium I consisted of L15 with 10 mmol · l^{-1} HEPES, 5.5 mmol · l^{-1} glucose and 1.0% (w/v) BSA; incubation medium II consisted of incubation medium I without BSA; incubation medium III consisted of incubation medium II with cycloheximide (30 mmol · l^{-1}) which resulted in no change in pH. Incubation media contained $NaHCO_3$ at a concentration of 2 mmol · l^{-1}.

Isolation. The isolation procedure for trout hepatocytes was modified from Moon et al. (1985). The fish was killed by a sharp blow on the head and transection of the spinal cord. The posterior portal vein was immediately cannulated (dia. 1.02 mm) and the liver was perfused (LKB 2120, Sweden) with perfusion buffer B at a rate of 4–7 ml · min^{-1}. Before the perfusion started the heart was punctured to prevent damage due to a pressure build up in the liver. After 5–10 min 35 ml perfusion buffer C was perfused at the same rate. After a further 5–10 min the perfusion was terminated and the liver carefully removed from the body, disconnected from the gall bladder and placed in 15 ml perfusion buffer C. The liver was chopped using scissors. After a further 10 min the mixture of isolated hepatocytes and non-digested liver was filtered through a nylon gauze (pore dia. 0.22 mm). The hepatocytes were washed three times in washing buffer I and centrifuged at 50 g for 2 min at 4 °C. The hepatocytes were then resuspended in 40 ml incubation medium I and kept in a shaking (100 rpm) water bath at 10.0 °C for 1 h. Hepatocyte concentration was determined with a cell cytometer. Cell viability was determined by 0.4% (w/v) trypan blue exclusion (Campbell et al. 1983). Further viability tests were carried out in respiratory tests by adding 20 mmol succinate · l^{-1} to isolated hepatocytes (see below). All isolations were performed between 10:00 and 11:00 a.m.

Effect of BSA on the viability of isolated trout hepatocytes. To determine the effect of 1% (w/v) BSA on the viability of the isolated hepatocytes, the cells were isolated and incubated with or without BSA. One group of fish ($n = 7$) was perfused and incubated without BSA, the second group ($n = 5$) was perfused without and incubated with BSA, and the third group ($n = 12$) was perfused and incubated in the presence of BSA. The isolation and incubation procedures were as described above. Viability was determined by trypan blue exclusion 1 h after isolation.

Incorporation studies. After the hepatocytes had been incubated in incubation medium I for 1 h they were washed three times in washing buffer II and resuspended in incubation medium II to a concentration of 12 · 10^6 hepatocytes · ml^{-1} for the incorporation studies. All incorporation studies were carried out in a total volume of 2.5 ml incubation medium II. Hepatocytes to which cycloheximide was added to inhibit protein synthesis were suspended in 2.5 ml incubation medium III. All treatments were performed in duplicate, i.e. from each liver two batches of cells were used as controls, two batches for cycloheximide treatment and two batches for O_2 consumption determinations. Incorporation studies were carried out using [^{3}H]PHE in the flooding-dose technique of Garlick et al. (1980). Hepatocytes were incubated in plastic 50-ml flasks (Nunclon, Denmark) in a shaking (100 rpm) water bath. Incorporation was measured at 5.0 (n=4), 10.0 (n=9), 14.5 (n=3), 17.5 (n=4) or 20.0 °C (n=2). Hepatocytes from one fish (n=number of fish) were used at one temperature. After a 10-min incubation a flooding dose of 50 µl 150 mmol · l^{-1} PHE with [^{3}H]PHE at 3.7 MBq · ml^{-1} (100 µCi · ml^{-1}) was added to the hepatocyte suspension and left for 40 min except when a time-course experiment was performed (see below). At the end of the incorporation period the hepatocytes were transferred into centrifuge tubes on ice and centrifuged at $50 \times g$ for 2 min at 4 °C after which the incubation medium was removed. Then 2 ml 0.5 mol · l^{-1} PCA was added to the hepatocyte pellet and the precipitate mixed and spun down at $500 \times g$ for 20 min at 4 °C. Three successive washes with 2 ml 0.5 mol · l^{-1} PCA were performed at $6000 \times g$ for 10 min at 4 °C to release the free [^{3}H]PHE. Samples were then resuspended in 0.3 mol · l^{-1} NaOH and protein determinations were carried out by the method of Lowry et al. (1951) using BSA as a standard. RNA determinations were subsequently carried out using the method of Munro and Fleck (1966). Determination of the specific radioactivities of the incubation medium and the protein-bound PHE were then determined using the method described by Garlick et al. (1980). k_s as a percentage of the protein mass synthesized per day was calculated as described earlier by Houlihan et al. (1988a). Also for each batch of isolated hepatocytes absolute protein synthesis rates as nanograms of protein synthesis per milligram of hepatocyte protein per minute were calculated. k_{RNA}, an indication of the synthesis rate per unit of RNA, was calculated as $\frac{10 \cdot k_s}{\text{RNA-to-protein ratio } (\mu g \cdot mg^{-1})}$ (Preedy et al. 1988).

Time-course of incorporation. Incorporation of [^{3}H]PHE was performed at 10.0 and 14.5 °C in a time-course experiment. Six flasks were incubated each with 30 · 10^6 cells per 2.5 ml. Three flasks contained incubation medium II, the other three, incubation medium III. After 10 min a flooding dose of 50 µl 150 mmol · l^{-1} [^{3}H]PHE was added to each individual flask. Immediately, one flask with and one without cycloheximide-treated cells were transferred to centrifuge tubes and centrifuged and processed as described above. The procedure was repeated at 20 and 40 min after the addition of PHE.

Effect of declining PO_2 *on protein synthesis.* Incorporation studies were performed under different PO_2 levels at 10.0 °C. Three air/N_2 mixtures were made in duplicate using a Wösthoff gas mixing pump: a 20, a 10 and a 5 kPa PO_2 mixture. Incubation flasks

containing the 2.5 ml hepatocyte suspension were gassed for 3 min while the flooding dose of 50 μl [^{3}H]PHE was added during the last minute of gassing, after which the flasks were sealed air-tight and placed in the shaking water bath. The hepatocytes were incubated for 40 min and processed as described above.

Respiration rates. Respiration rates of isolated trout hepatocytes were measured using a Rank Oxygen Electrode (System model 10, Cambridge, England) connected to a Linseis pen recorder. Respiration rates were measured at 5.0, 10.0, 14.5, 17.5 and 20.0 °C in a total volume of 2.5 ml incubation medium II. The O_2 electrode was calibrated using air- and N_2-saturated incubation medium II at the desired temperature. Blank determinations of O_2 consumption using incubation medium II without the cells were performed. After the hepatocytes had been incubated in incubation medium I for 1 h, they were washed three times in washing buffer II and resuspended in incubation medium II for the respiration experiment. This part of the experiment started simultaneously with the incorporation experiment. A total of $30 \cdot 10^6$ hepatocytes in 2.5 ml air-saturated incubation medium II were added to the respiration chamber and sealed with an air-tight stopper. Within 1 min after sealing the chamber, a linear decline of PO_2 with time was recorded. Between a PO_2 of 16 and 13 kPa, the seal was removed and cycloheximide added to a final concentration of 30 mmol $\cdot$ l^{-1} after which the seal was replaced. Within 5–8 min after adding cycloheximide the decline of PO_2 in time approached a lower constant level. The experiment was terminated when the PO_2 reached 10 kPa. The seal was removed and the hepatocytes recovered in a centrifuge tube using a pipette. The chamber was washed with 2.5 ml incubation medium II to collect the remaining hepatocytes. The hepatocytes were then centrifuged for 2 min at $50 \times g$ and 4 °C and the protein and RNA content determined as described. For every isolation a succinate exclusion test was performed to establish the viability of the hepatocytes. This was carried out with $30 \cdot 10^6$ cells per 2.5 ml in incubation medium II or incubation medium III added to the respirometer chamber which was sealed as described above. After a 5-min linear decline of PO_2, the seal was removed and succinate (20 mmol $\cdot$ l^{-1} final concentration) was added. The chamber was then sealed again and respiration rate monitored. Separate experiments were carried out in which ouabain (1 mmol $\cdot$ l^{-1}) was used to inhibit the maintenance of Na^+/K^+-ATPase (Bianchini et al. 1988). Results were expressed as nmoles of O_2 consumption per minute per milligram of hepatocyte protein. The decline of PO_2 with time in 2.5 ml incubation medium was transformed into nanomoles of O_2 by using the O_2 content of 2.5 ml air-saturated incubation medium II at each temperature. The total O_2 content of air-saturated incubation medium II was measured at each temperature using the method of Tucker (1967).

Statistics. Student's *t*-test with a 5% level of confidence was used throughout unless otherwise stated.

Results

Hepatocyte viability

Hepatocyte yield was on average 250–400 $\cdot 10^6$ cells per liver, with less than 1% red blood cel contamination. Addition of 1% (w/v) BSA to the perfusion buffer, washing buffer and incubation medium improved the viability of the isolated hepatocytes both during and after isolation; there was a significant increase in trypan blue exclusion when BSA was added to perfusion buffer II and incubation medium I. However, BSA also increased the total amount of protein of the hepatocytes, reducing the RNA-to-protein (μg $\cdot$ mg^{-1}) ratio significantly ($P < 0.01$) from 85.78 ± 11.99 ($n = 13$) to 43.87 ± 22.51 ($n = 8$). As the respiration and protein synthesis results were expressed per milligram of cellular protein, the presence of BSA would underestimate hepatocyte O_2 consumption and protein synthesis. It was assumed that BSA was attached to hepatocytes since three washes with washing buffer II resulted in the restoration of in vivo RNA-to-protein ratios. The removal of BSA before the O_2 consumption and protein synthesis measurements did not alter the trypan blue staining of the hepatocytes during the 40 min of respiration and incorporation experiments. The three washes before the experiments were therefore included in the method of isolation.

Mean values for trypan blue exclusion were 93.8 ± 3.0% in all the experiments ($n = 26$). No hepatocyte preparations with less than 90% exclusion are included in the results. Further viability tests were performed using the succinate exclusion test (Bauer et al. 1975; Mapes and Harris 1975; Campbell et al. 1983; Seibert 1985). It was found that the respiration rate of normal, cycloheximide- and ouabain-treated cells did not increase by more than 5% after addition of succinate. Trypan blue exclusion after cycloheximide treatment was always better than 90%.

An indication of metabolic stability of hepatocytes during the incorporation and respiration was assessed by measuring O_2 consumption rates from 0 to 20, 20 to 40, and 40 to 60 min after the incorporation studies had started. There was no significant change in respiration rates within 1 h after the experiment was initiated. Respiration and incorporation experiments were completed 40 min after the experiments were initiated.

Time-course of incorporation

The reliability of the method of measuring protein synthesis was determined by following the PHE specific radioactivity of the incubation medium and the rate of labelling of the protein. The mean radioactivity of the incubation medium remained constant at 1080 dpm $\cdot$ nmol PHE^{-1} for the 40-min incubation period (Fig. 1a). Figure 1b shows that the protein-bound specific radioactivity (dpm $\cdot$ nmol PHE^{-1}) increases linearly with time at both 10 and 14.5 °C.

Effect of PO_2 *on respiration and protein synthesis*

Figure 2 illustrates the effect of declining PO_2 on the respiration and protein synthesis of isolated hepatocytes. It was found that the rate of O_2 consumption did not change between 20.0 and 4.0 kPa PO_2. Below 4.0 kPa the O_2 consumption decreased dramatically, almost down to zero. From this it can be concluded that the range over which respiration and incorporation were measured (20–10 kPa) did not influence respiration rates. To check whether synthesis rates were constant between 20 and 10 kPa, the synthesis of protein in trout hepatocytes was expressed as disintegrations per minute per milligram of cellular protein for a PO_2 of 20, 10 and 2 kPa (Fig. 2). The rate of incorporation was found to be constant between 20 and 10 kPa but started to decline at around 2 kPa.

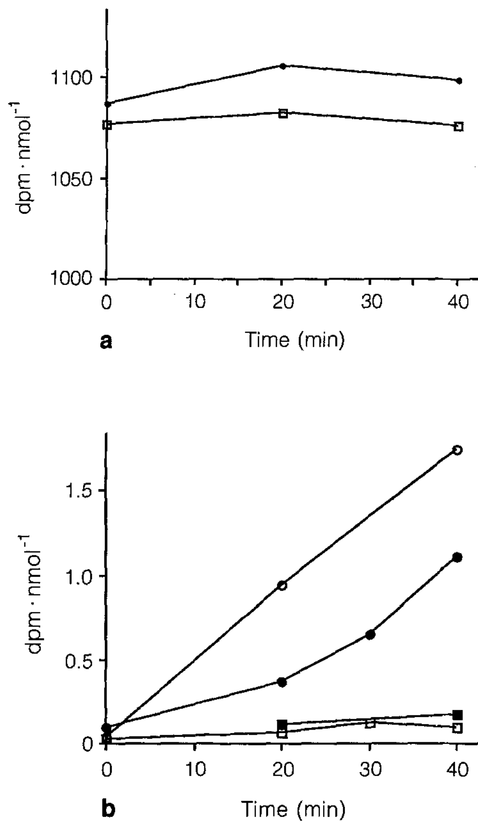

Fig. 1. **a** Time-course of incubation medium phenylalanine specific radioactivity (dpm · nmol phenylalanine^{-1}) at 10 °C (●) and for cycloheximide-treated cells at 14.5 °C (□). **b** Time-course of incorporation of [^{3}H]PHE into hepatocyte cellular protein for cells at 10 °C (●) and 14.5 °C (○), and for cycloheximide-treated cells at 10 °C (–□–) and 14.5 °C (–■–)

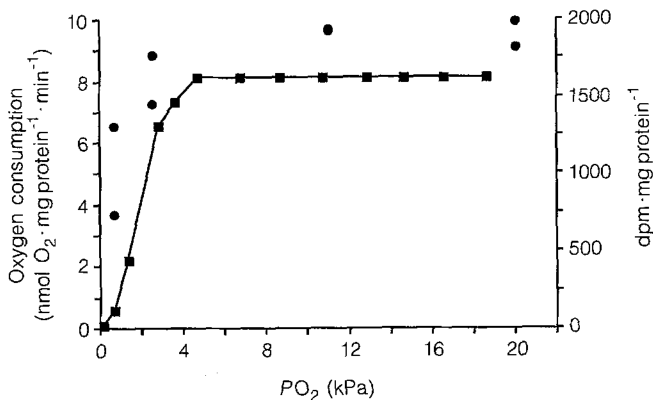

Fig. 2. Effect of PO_2 on protein synthesis (●) and O_2 consumption (■) at 14.5 °C

Effect of temperature on incorporation and O_2 consumption

A range of different acute incubation temperatures were included to induce variable protein synthesis and O_2 consumption values (Fig. 3a, b). The Q_{10} of the mean O_2 consumption values between 5.0 and 17.5 °C was 2.38. The mean O_2 consumption values at 5 °C are significantly different from those between 10.0 and 17.5 °C. The Q_{10} for rates of protein synthesis were 2.34 between 5.0 and 17.5 °C. Mean protein synthesis rates at 5 °C are significantly different from that at all other temperatures. Significant differences were observed between synthesis rates at 10.0 and 14.5 °C and at 10.0 and 17.5 °C.

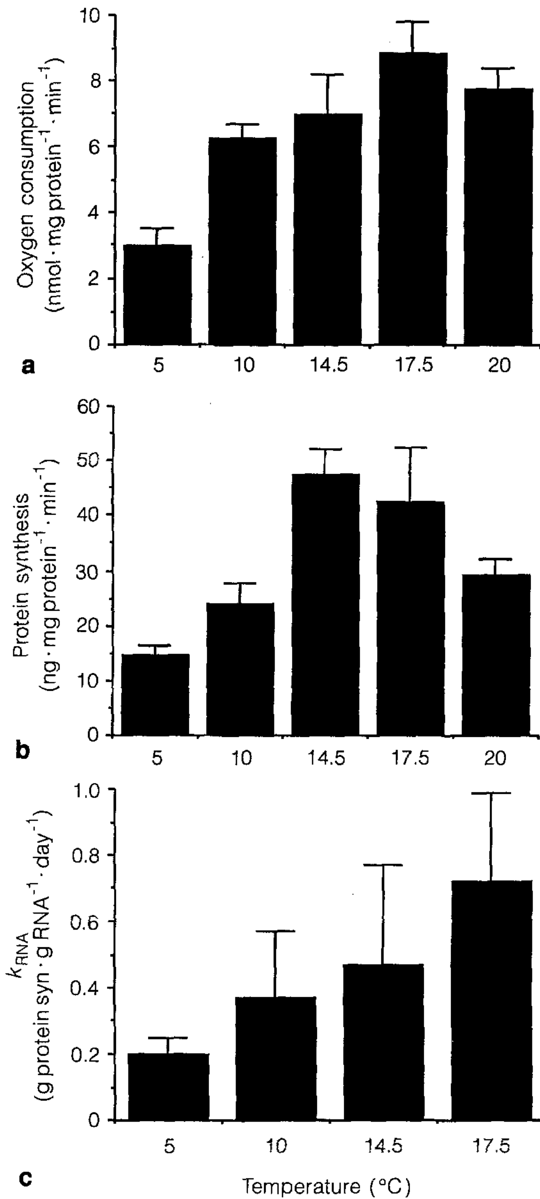

Fig. 3. **a** Effect of acute temperature incubation on O_2 consumption at 5, 10, 14.5, 17.5 and 20 °C. **b** Effect of temperature on protein synthesis at 5, 10, 14.5, 17.5 and 20 °C. **c** Effect of temperature on k_{RNA} at 5, 10, 14.5 and 17.5 °C

RNA protein ratios were not significantly different between the groups of hepatocytes (mean 85.78 ± 11.99 μg RNA · mg protein^{-1}, $n = 13$). Therefore, calculation of k_{RNA} revealed an increase from 5.0 to 17.5 °C (Fig. 3c), indicating that the increase in protein synthesis due to the acute temperature increase was a result of increased synthesis per unit of RNA. At 20 °C the RNA to protein ratios were not measured, but using the mean values k_{RNA} at this temperature can be calculated as 0.49 g synthesized · g RNA^{-1} · day^{-1}.

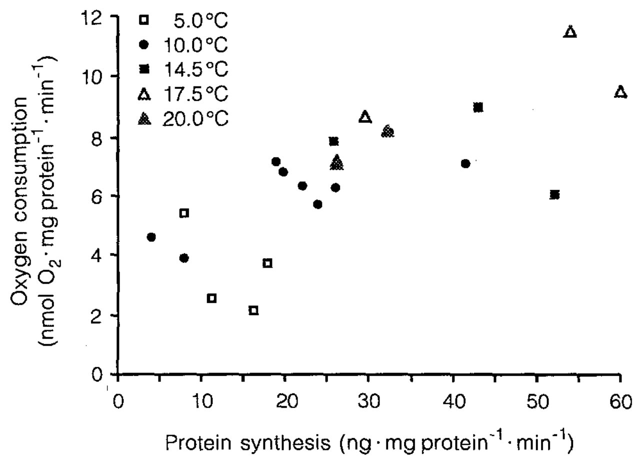

Fig. 4. Relation between protein synthesis of hepatocytes and their O_2 consumption (nmol $O_2 \cdot$ mg protein$^{-1} \cdot$ min^{-1}) at 5, 10, 14.5, 17.5 and 20 °C

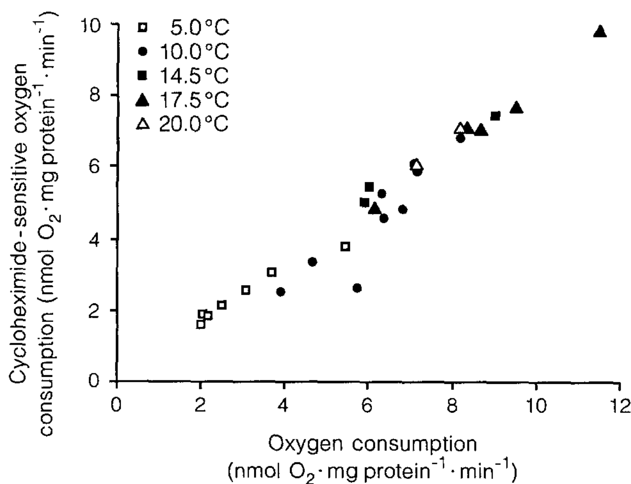

Fig. 5. Relation between total respiration and cycloheximide-sensitive respiration for cells incubated at 5, 10, 14.5, 17.5 and 20 °C

The rate of O_2 consumption of hepatocytes increased with increasing protein synthesis rate up to approximately 20–30 ng protein synthesis $\cdot$ min$^{-1} \cdot$ mg cellular protein^{-1} (Fig. 4). Thereafter, O_2 consumption does not appear to increase as rapidly with increasing protein synthesis rates.

Comparison of cycloheximide-sensitive O_2 consumption, total O_2 consumption and protein synthesis

Cycloheximide had a lag period of 5–10 min before the respiration rate reached a lower constant level. The decrease in O_2 consumption after addition of cycloheximide is regarded as the reduction in O_2 consumption required to fulfil amino acid elongation during protein synthesis. The total O_2 consumption rate was plotted against the cycloheximide-sensitive O_2 consumption (Fig. 5) of cells from one fish. The cycloheximide-sensitive O_2 consumption was 79.7 ± 9.5% ($n = 26$) of the total respiration for all hepatocyte isolations. There was no clear indication that cycloheximide inhibition of O_2 consumption was different at different temperatures.

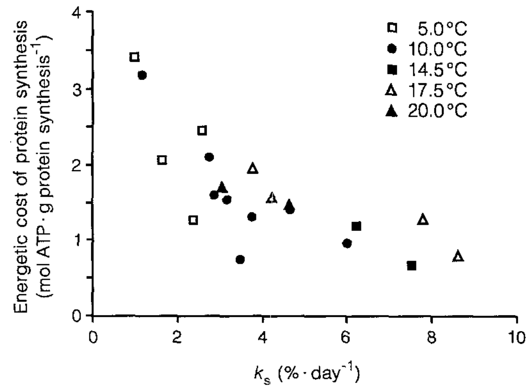

Fig. 6. Relation between the energetic cost of cycloheximide-sensitive protein synthesis and the fractional rate of protein synthesis measured at 5, 10, 14.5, 17.5 and 20 °C

At 5 °C a 5-min pre-incubation with cycloheximide followed by a 40-min incubation with cycloheximide brought about a 54.5% reduction in protein synthesis rate, whereas at the other temperatures (Fig. 1), protein synthesis inhibition ranged from 83.5% (20 °C) to 88.2% (14.5 °C). The mean inhibition was 79.9 ± 2.83%. At lower cycloheximide concentrations (8.9 mmol $\cdot$ l^{-1}) the inhibition of protein synthesis had not occurred within 8 min after addition and only 50–75% inhibition was finally achieved.

The average decrease of O_2 consumption in 1 mmol $\cdot$ l^{-1} ouabain was 2.8 ± 2.8% ($n = 6$) in incubation medium II. The decrease in O_2 consumption was achieved within 5 min.

Energetic cost of protein synthesis

To relate the amount of O_2 consumption attributed to protein synthesis and the synthesis rate, the two are compared after inhibition of protein synthesis with cycloheximide. The energetic cost of protein synthesis was expressed as nanomoles of cycloheximide-sensitive O_2 consumption per nanogram of cycloheximide-sensitive protein synthesized. The O_2 consumption related to protein synthesis was transformed into moles of ATP on the assumption that 1 mol O_2 is equivalent to 6 mol ATP (Reeds et al. 1985). The result was a wide range of different costs (moles ATP per gram of protein synthesis) between hepatocytes of different fish with increasing fractional rates of protein synthesis (k_s; Fig. 6). The data give a significant correlation in the form $\log y = 0.497 - 0.593 \log x$ (x, fractional rate of protein synthesis; k_s, % $\cdot$ day^{-1}; y is the energy cost of synthesis; $n = 20$; $r^2 = 0.66$; $P < 0.001$). When the data at 10 °C only are subjected to the same analysis the regression equation is: $\log y = 0.532 - 0.724 \log x$ ($n = 8$; $r = 0.646$; $P < 0.01$). Therefore, there is evidence from the data at a single temperature and from all the data from different temperatures that the cost of protein synthesis declines in a non-linear fashion with increasing k_s.

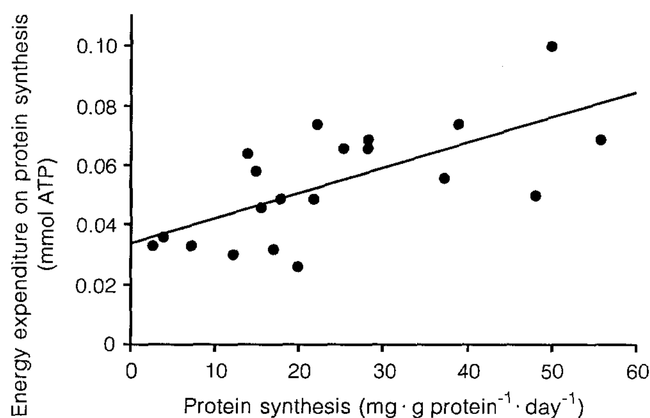

Fig. 7. Relation between the energy expenditure related to cycloheximide sensitive protein synthesis and the rate of protein synthesis sensitive to cycloheximide. Standardization for 1 g cellular protein and for 24 h

This result stimulated an analysis of the energetic cost of protein synthesis into a fixed and a variable cost. Therefore, the costs in terms of moles of ATP per gram of protein synthesized were standardized for 1 g protein mass and 24 h. Multiplication of the cost of protein synthesis (moles of ATP per gram of protein synthesized) with the synthesis rate (grams synthesized per gram of protein mass per day) gave the total energy expenditure (moles of ATP) attributed to protein synthesized at that specific rate of protein synthesis. The result (Fig. 7) is a significant linear relation between the rate of protein synthesis (x, milligram synthesized per gram of protein mass per day) and the energy expenditure related to protein synthesis (y, millimoles of ATP) which can be described by the equation: $y = 35.61 + 0.812x$ ($n = 20$; $r^2 = 0.45$; $P < 0.001$). The intercept was 35.61 ± 1.37 mmol ATP ($n = 20$). This suggests that there is a fixed energy expenditure of 32.04 mmol ATP · g protein synthesized^{-1}, which is independent of the amount of protein synthesized per day.

Discussion

The isolated hepatocytes used in this study appear to be comparable to those used in previous studies in terms of oxygen consumption, trypan blue exclusion and appearance under the electron microscope (results not included). Cycloheximide has been found to be tolerated by fish when injected in vivo, at least in the short term (Nichols and Fleming 1990). The concentrations used here were higher than those used in other studies [e.g. 0.5 mmol · l^{-1}; Hasselgren et al. (1990)] but the effects were rapid and high levels of inhibition were achieved.

Fractional rates of protein synthesis in trout liver in vivo have been reported to be around 15% · day^{-1} in continuously fed rainbow trout (McMillan and Houlihan 1988, 1989; Foster et al. 1991) at around 10 °C. The isolated hepatocytes in this study did not achieve this fractional rate of protein synthesis; the value from the present study at 10 °C is 3.5% · day^{-1}. The hepatocytes were prepared from livers taken from larger animals than those used in previous studies, and a decline in protein synthesis with increasing body size could therefore be expected (Houlihan et al. 1986). However, the isolated cells are likely to be exhibiting the lower in vitro rates of protein synthesis characteristic of many in vitro preparations (Houlihan et al. 1988b).

It has been demonstrated in this study that isolated trout hepatocytes use on average 79.7% of their total O_2 consumption on cycloheximide-sensitive protein synthesis. Compared to whole-animal studies this is a high percentage: Aoyagi et al. (1988) found in whole chickens that on average only 28.8% of the total heat production accounted for cycloheximide-sensitive protein synthesis; in whole cattle it has been calculated that around 37% of the total energy expenditure can be accounted for by protein synthesis (Lobley et al. 1980). However, the energy expenditure for protein synthesis in muscle was found to be only 2.0–3.3% of the total energy expenditure (Gregg and Milligan 1982). These results support the hypothesis (Harris et al. 1989) that tissue-specific protein synthesis rate and protein synthesis-related O_2 consumption are associated.

The high proportion of the energy budget for protein synthesis in hepatocytes indicates that not much energy is left for other purposes. One of the other main energy-demanding processes at the cellular level is the maintenance of Na^+/K^+ levels via the Na^+/K^+-ATPase pump. Rat diaphragm muscle Na^+/K^+ transport accounts for 26.3% of the total energy expenditure (Gregg and Milligan 1979), and in calf muscle this accounted for 40% (Gregg and Milligan 1982). However, perfused rat liver (Folke and Sestoft 1977) and adipose tissue (Chinet et al. 1977) use no more than 6% of the total energy expenditure for the maintenance of Na^+/K^+ transport. The average value of 2.8% found for isolated trout hepatocytes in this study is therefore in accordance with the low levels found in rat liver.

Together, the energy needed for protein synthesis and Na^+/K^+ transport accounts on average for 82.5%, so that 17.5% of the total energy expenditure remains for other purposes (e.g. gluconeogensis, lipogenesis). The ability of the hepatocyte to maintain normal O_2 consumption and protein synthesis rates over a wide range of PO_2 possibly relates to the low PO_2 levels in incoming blood from the portal vein.

The energetic cost of protein synthesis in fish has been reviewed by Jobling (1985) and Houlihan (1991). As discussed in these reviews a correlation exists between metabolic rate and protein synthesis which is often used to express the cost of protein synthesis. However, simultaneous measurements of metabolic rate and protein synthesis are scarce. The published "correlative" cost values show great variability and are mostly based on mammalian experimental models (Table 1). The situation is further complicated by the suggestion that different tissues have different energetic costs for protein synthesis (Gregg and Milligan 1982; Adeola et al. 1989; Harris et al. 1989; McBride and Early 1989). In the present study the relation between protein synthesis and O_2 consumption was non-linear over the range of synthesis rates measured, although all cells utilized the same

Table 1. Summary of the energetic costs for protein synthesis as calculated from the available literature

Species	k_s (% · day^{-1})	ATP (mmol · g protein synthesized^{-1})	Method used	Ref[a]
General	–	50–100	Theoretical	[1]
Calf muscle	0.7–1.5	2700	Correlation	[2]
Pig muscle	1.3–6.0	980–3450	Correlation	[3]
Trout hepatocytes	1–8.5	223–830	Correlation	[4]
Whole sheep	3.1–5.2	292	Correlation	[5]
Mytilus edulis	3.1–7.9	148	Correlation	[6]
Sheep hepatocytes	30–50	42	Correlation	[7]
Chickens	29	72	Inhibition with cycloheximide	[8]
Trout hepatocytes	1–8.5	670–3500	Inhibition with cycloheximide	[4]

[a] [1] Jobling 1985; [2] Gregg and Milligan 1982; [3] Adeola et al. 1989; [4] This study; [5] Harris et al. 1989; [6] Hawkins et al. 1989; [7] McBride and Early 1989; [8] Aoyagi et al. 1988

percentage of their total O_2 consumption for protein synthesis.

To express this point more clearly one must look at the fractional synthesis rates of isolated sheep hepatocytes at 37 °C (McBride and Early 1989) which synthesize at a rate of 30–50% · day^{-1}, 6–8 times higher than the isolated trout hepatocytes at 10 °C. However, the rate of O_2 consumption for sheep and rat hepatocytes (Howard and Pesch 1968; Howard et al. 1973; McBride and Early 1989) is not much further increased when compared with trout hepatocytes synthesizing at 7–8% · day^{-1}. This would indicate that the correlative cost decreases with increasing synthesis rate.

A similar conclusion of a changing cost of protein synthesis comes from direct measurements using cycloheximide to inhibit protein synthesis. Unfortunately, there is no reference in the mammalian literature to measurements of the cost of protein synthesis in isolated hepatocytes using the methodology adopted in this study. The only reference available (Aoyagi et al. 1988) is on 1-week-old whole chickens; with a fractional synthesis rates five times higher than in isolated trout hepatocytes the measured cost in moles of ATP per gram of protein synthesis decreases (Table 1). The energetic cost of protein synthesis in hepatocytes declines with increasing synthesis rate from about 3500 mmole ATP · g^{-1} at 0–1% · day^{-1} to a minimum value of 670 mmol ATP per g protein synthesized at a k_s of 7.4% · day^{-1} (Fig. 6). The cost for protein synthesis in chickens was 72 mmol ATP per g protein synthesized, measured at a fractional synthesis rate of 29% · day^{-1}. Using the data in Fig. 6, the costs of protein synthesis of trout hepatocytes at this fractional protein synthesis rate would be 426 mmol ATP per g protein synthesized.

It is remarkable that a declining energetic cost of protein synthesis within increasing rates of synthesis can be found in the protein synthesis costs quoted in the literature (Table 1). All the evidence therefore points to the likely hypothesis that the cost of protein synthesis is composed of a fixed cost (independent of synthesis rate) and a variable cost component; therefore, at low synthesis rates the cost per unit of synthesis is very high, as a large part of the energy expenditure consists of the fixed energy expenditure. What these fixed cost components are remains speculative, but constant activation of tRNA as well as the production of rRNA, which remains remarkably constant regardless of the rate of protein synthesis (Oka et al. 1989), very probably form a part of this fixed energy expenditure. It has been suggested by Cooper and Gibson (1971) that accumulation of rRNA in the cytoplasm occurs only at half the rate at which it is synthesized in the nucleus. This would mean that the cost of rRNA synthesis would be underestimated by at least 100%.

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