

## CONTINUOUS TRACER INFUSION TO MEASURE *IN VIVO* METABOLITE TURNOVER RATES IN TROUT

FRANÇOIS HAMAN AND JEAN-MICHEL WEBER\*

*Biology Department, University of Ottawa, 30 Marie Curie, Ottawa, Ontario, Canada K1N 6N5*

*Accepted 16 January 1996*

### Summary

This paper describes a double dorsal aorta catheterization technique allowing the measurement of substrate turnover rates by continuous infusion of metabolic tracers in rainbow trout. The placement of both catheters can be performed in about 30 min with minimal surgical training. As a practical example of a routine substrate flux measurement, glucose turnover rate of resting trout was measured by primed continuous infusion of 6-<sup>3</sup>H]glucose through one of the catheters and blood sampling from the other. The animals maintained resting metabolic rate, normal blood glucose and low blood lactate concentrations throughout the experiments. Glucose isotopic steady state was achieved in less than 40 min, and mean turnover rate was  $9.0 \pm 0.7 \mu\text{mol kg}^{-1} \text{min}^{-1}$  ( $N=8$ ). Comparison with published glucose turnover rates measured in trout and other teleost species suggest that

values previously obtained using the 'bolus injection technique' are underestimates of true flux rates. We conclude that the simple surgical technique presented here opens the door to the dynamic study of substrate kinetics under a variety of experimental conditions and that it can be adapted to the investigation of most metabolic substrates, including fatty acids, glycerol, amino acids and lactate, in addition to glucose. Future application of the continuous infusion technique under steady-state as well as non-steady-state conditions will add a new dimension to the general understanding of fish metabolism.

Key words: substrate fluxes, metabolite replacement rate, hepatic glucose production, continuous infusion, non-steady-state kinetics, glucose, lactate, glycerol, fatty acids, amino acids, tracer methodology, *Oncorhynchus mykiss*.

### Introduction

Reliable methods to measure metabolite turnover rates *in vivo* are widely available (Hetenyi *et al.* 1983), and they have been used extensively in mammalian metabolic studies, especially for humans (Wolfe, 1992). In comparison, surprisingly little work has been carried out on fish (Garin *et al.* 1987; Weber and Zwingelstein, 1995), mainly because it has been difficult to adapt tracer techniques to aquatic animals of smaller relative body size. The *bolus injection* method has been used almost exclusively in fish studies (Weber and Haman, 1995) because it only requires a single catheter and can consequently be performed with a simple dorsal aortic cannula. This method has serious limitations, however, and it has been almost totally abandoned for mammals because it can only be used under steady-state conditions and each experiment only allows the calculation of a single turnover rate after analysis of a minimum of 6–10 blood samples. In mammals, metabolite turnover rates are quantified using the more versatile *continuous infusion* technique because it allows measurements under steady-state as well as non-steady-state conditions; also, much more information can be obtained from a single experiment because instantaneous flux can be calculated from each blood sample separately. Unfortunately,

this method requires the surgical placement of two catheters – one to infuse the metabolic tracer, the other for blood sampling – and it has never been adapted to investigate the metabolism of trout, the most commonly used teleost model.

The now classic dorsal aorta cannulation technique of Smith and Bell (1964), later modified by Soivio *et al.* (1975), revolutionized the study of fish metabolism by allowing repeated blood sampling and intravascular injections in undisturbed, non-anaesthetized fish. Over the last 30 years, dorsal aorta cannulation has been used routinely to investigate fundamental aspects of fish biochemistry and physiology. This surgical approach has provided invaluable information on the effects of various stresses including exercise, changes in water temperature, pH, salinity, oxygen content and toxicant concentration. In studies of fish metabolism, the blood concentration of key metabolites has been monitored to examine the biochemical consequences of these stresses. In this context, turnover rate is a much more informative parameter than concentration, and the temptation to use concentration changes to draw conclusions about fluxes has not always been resisted. Such extrapolation is clearly unwarranted, however, because flux and concentration do not

necessarily change in parallel (Wolfe, 1992); a very large change in turnover rate can be associated with an increase, no change or even a decrease in concentration, depending on the prevailing difference between rates of appearance and disappearance. Here, therefore, our goals were to develop an easily performed double catheterization for rainbow trout and to use the continuous tracer infusion technique to quantify resting glucose turnover as a practical example.

## Materials and methods

### Animals

Rainbow trout *Oncorhynchus mykiss* (Walbaum) of both sexes (831–1265 g) were purchased from Linwood Acres Trout Farm (Campbellcroft, Ontario, Canada) and held in 5501 flow-through tanks at 15 °C. They were kept in dechloraminated, well-oxygenated Ottawa tap water under a 12 h:12 h light:dark photoperiod. The animals were acclimated to these conditions for at least 1 month before experiments and they were fed Purina trout chow three times a week until satiation.

### Placement of catheters

24 h before isotope infusion, each animal was anaesthetized in 0.1 g l<sup>-1</sup> ethyl-*N*-aminobenzoate sulphonic acid (MS-222) buffered with 0.2 g l<sup>-1</sup> bicarbonate. During surgery, the anaesthetic solution was recirculated and aerated to perfuse the gills. The cannulation technique of Soivio *et al.* (1975) was modified to allow the placement of two separate PE-50 catheters (Intramedic, Clay-Adams) in the dorsal aorta. The first catheter was used for isotope infusion and it was inserted at the third gill arch before being fed 8–10 cm caudally into the vessel. The second catheter was used for blood sampling. It was inserted into the artery at the first gill arch and was fed caudally no more than 2 cm (see Fig. 1). These infusion and sampling sites provide an accurate measurement of glucose turnover because the determination of glucose kinetics does not depend on exact catheter placement (Katz, 1992; Norwich, 1992; Wolfe, 1992). Each catheter was filled with heparinized (10 i.u. ml<sup>-1</sup>) Cortland saline without glucose (Wolf, 1963) and held securely in place by two stitches to the roof of the mouth. After recovery from anaesthesia, each animal was placed in an opaque Plexiglas chamber (60 cm × 16 cm × 18 cm) supplied with the same quality water as the holding tanks at a rate of 5–6 l min<sup>-1</sup>. The chamber was sealed with a lid tightly

held in place with screws, and both catheters were allowed to exit the chamber through a small opening in the lid. All experiments were carried out between 24 and 48 h after surgery.

### Measurement of glucose turnover rate

While the fish was resting quietly in the Plexiglas chamber, a continuous infusion of 6-[<sup>3</sup>H]glucose (New England Nuclear, 1.6 TBq mmol<sup>-1</sup>; Amersham, 1.11 TBq mmol<sup>-1</sup>) was started with a calibrated syringe pump at 1 ml h<sup>-1</sup> (Harvard Apparatus, South Natick, MA, USA). Glucose tritiated in position 6 has been shown to yield the best estimate of glucose turnover because detritiation due to hepatic cycling is minimal (Katz, 1979). A priming dose equivalent to 90 min of infusion was injected as a bolus before starting the pump to decrease the time necessary to reach isotopic steady state in the relatively large glucose pool (Wolfe, 1992). The infusate was prepared daily by drying a sample of the stock solution under N<sub>2</sub> and redissolving it in Cortland saline without heparin. Exact infusion rate was determined for each experiment by counting a sample of the infusate; infusion rates ranged between 30 059 and 108 333 disintegrations min<sup>-1</sup> kg<sup>-1</sup> min<sup>-1</sup>. In the Results section, glucose specific activities have been divided by infusion rate for each experiment to provide standardized values allowing meaningful comparisons. Blood samples (300 µl each) were drawn after 40, 50 and 60 min of infusion. Each sample was diluted in 500 µl of perchloric acid (8%) and centrifuged. After separation, the perchloric acid extracts were used to measure glucose and lactate concentrations at 340 nm using standard enzymatic methods (Bergmeyer, 1985) on a Milton Roy spectrophotometer (Spectronic 1001). Glucose specific activity was measured by counting 100 µl of perchloric acid extract in 10 ml of ACSII scintillation fluid (Amersham, Oakville, Ontario, Canada). This procedure was used because total tritium activity has been shown to be restricted to glucose and water (Katz *et al.* 1974). Therefore, this simple technique to determine specific activity is used routinely for glucose turnover measurements in dogs (Moates *et al.* 1988), humans (Molina *et al.* 1990; Levy *et al.* 1989), birds (Marsh and Dawson, 1982) and fish (West *et al.* 1994a,b). Here, the drying step was eliminated because negligible amounts of <sup>3</sup>H<sub>2</sub>O were found in our experiments owing to the very high water rate turnover rate found in fish. Preliminary experiments showed that <sup>3</sup>H<sub>2</sub>O activity represented less than 3% of total activity,

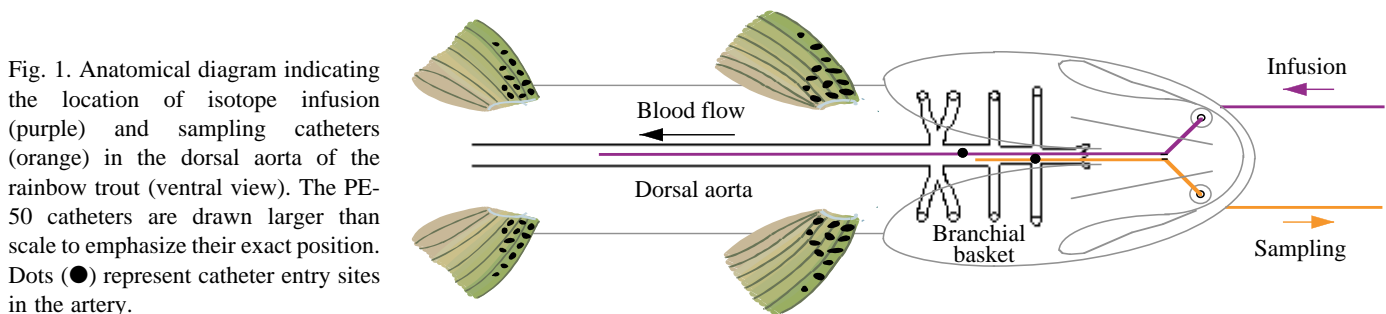


Fig. 1. Anatomical diagram indicating the location of isotope infusion (purple) and sampling catheters (orange) in the dorsal aorta of the rainbow trout (ventral view). The PE-50 catheters are drawn larger than scale to emphasize their exact position. Dots (●) represent catheter entry sites in the artery.

even after 3 h of 6-[<sup>3</sup>H]glucose infusion. Counting was performed on a Tri-Carb 2500 scintillation counter (Packard, Canada) with external quench correction. Glucose turnover rate ( $R_t$ ) was calculated with the steady-state equation of Steele (1959) and glucose clearance rate was determined by dividing turnover rate by glucose concentration.

#### Metabolic rate measurement

Oxygen consumption was measured by stopping the external water supply for three 10 min periods during each isotope infusion, and recycling the chamber water within a 15 l closed system. Particular care was taken to eliminate air bubbles within the system and to avoid exchange between the recirculated water and atmospheric air. Water flow rate through the chamber remained at 5–6 l min<sup>-1</sup> during the metabolic rate measurements. During each 10 min interval, O<sub>2</sub> concentration was recorded every minute with a calibrated oxygen electrode (Oxyguard, Handy MK III, Valox Ltd). The total decrease in  $P_{O_2}$  never exceeded 10% of saturation  $P_{O_2}$  but it was large enough to measure oxygen consumption accurately. The total volume of recirculated water (15 l) was selected to provide an optimal ratio of fish size to respirometer volume (Steffensen, 1989). The rate of oxygen consumption ( $\dot{M}_{O_2}$ ) was calculated using the closed-system equation of Steffensen (1989). After each metabolic rate measurement, return to saturation  $P_{O_2}$  was accomplished by flushing the system for 5 min. All results are given as means  $\pm$  S.E.M.

## Results

### Catheterization

The double cannulation technique proposed in this paper is summarized in Fig. 1 where exact catheter placements are indicated. Complete surgery was achieved in 25–40 min depending on the individual fish (where surgery time is the time elapsed between starting the anaesthesia and complete recovery with the animal having regained its normal balance).

Surgical success rate was higher than 90% with the large rainbow trout used here (see Table 1). Additional experiments (results not shown) revealed that it is possible to cannulate smaller animals (down to 600 g) with the same success rate; however, performing this double catheterization in animals smaller than 500 g became increasingly difficult because of the lack of working space within the mouth. The surgical procedure occasioned minimal blood loss, as indicated by the maintenance of high haematocrit values (Table 1). After removing catheterized fish from the anaesthesia solution, recovery occurred in 5–10 min.

### Continuous isotope infusion

Both catheters were kept patent between surgery and isotope infusion without problems. The opaque Plexiglas chamber, low infusion rate (1 ml h<sup>-1</sup>) and good sampling catheters allowed isotope infusion and blood sampling to occur without disturbing the fish, as indicated by the normal resting metabolic rates measured during the experiments ( $\dot{M}_{O_2}$  ranged between 33.5 and 63.3  $\mu\text{mol O}_2 \text{ kg}^{-1} \text{ min}^{-1}$ , and it averaged 42.2 $\pm$ 3.7  $\mu\text{mol O}_2 \text{ kg}^{-1} \text{ min}^{-1}$ , see Table 1). In addition, no thrashing or unusual movements were observed as a result of isotope infusion or blood sampling; low blood lactate and normal blood glucose concentrations were maintained throughout the infusion procedure (Table 1).

### Glucose kinetics

The infusion rates, priming dose and blood sampling schedule selected here were adequate to achieve concentration and isotopic steady-state conditions for glucose in 40 min of infusion. Glucose concentration, specific activity and turnover rate did not change significantly over time after 40 min of infusion (Fig. 2,  $P > 0.7$ , ANOVA). Results for individual infusion experiments are presented in Table 1. The resting glucose turnover rate  $R_t$  of rainbow trout ranged between 6.2 and 11.8  $\mu\text{mol kg}^{-1} \text{ min}^{-1}$  and it averaged 9.0 $\pm$ 0.7  $\mu\text{mol kg}^{-1} \text{ min}^{-1}$ . Turnover rate and concentration

Table 1. Metabolic rate ( $\dot{M}_{O_2}$ ), blood glucose concentration, turnover rate ( $R_t$ ) and clearance rate in resting, post-absorptive rainbow trout during continuous infusions of 6-[<sup>3</sup>H]glucose at 15°C

Experiment	$M_b$ (g)	Hct (%)	[Lactate] (mmol l <sup>-1</sup> )	$\dot{M}_{O_2}$ ( $\mu\text{mol kg}^{-1} \text{ min}^{-1}$ )	[Glucose] (mmol l <sup>-1</sup> )	$R_t$ ( $\mu\text{mol kg}^{-1} \text{ min}^{-1}$ )	Clearance rate (ml kg <sup>-1</sup> min <sup>-1</sup> )
1	1036	28	0.6 $\pm$ 0.11	40.0 $\pm$ 2.4	8.7 $\pm$ 0.1	11.8 $\pm$ 0.5	1.4
2	831	18	0.6 $\pm$ 0.02	40.4 $\pm$ 0.5	6.6 $\pm$ 0.1	9.5 $\pm$ 0.2	1.4
3	1265	25	0.6 $\pm$ 0.02	63.3 $\pm$ 2.7	3.6 $\pm$ 0.1	7.2 $\pm$ 0.3	2.0
4	831	21	0.6 $\pm$ 0.02	33.5 $\pm$ 0.8	6.3 $\pm$ 0.1	9.3 $\pm$ 0.2	1.5
5	915	32	1.6 $\pm$ 0.00	43.3 $\pm$ 1.0	3.9 $\pm$ 0.1	10.4 $\pm$ 0.4	2.7
6	1168	30	0.7 $\pm$ 0.07	35.1 $\pm$ 2.1	7.9 $\pm$ 0.1	6.9 $\pm$ 0.1	0.9
7	1011	36	2.3 $\pm$ 0.06	–	3.4 $\pm$ 0.1	6.2 $\pm$ 0.3	1.8
8	913	33	1.3 $\pm$ 0.12	40.0 $\pm$ 2.3	6.8 $\pm$ 0.1	10.8 $\pm$ 0.3	1.6
Mean	996	27.5	0.8	42.2	5.9	9.0	1.7
S.E.M. (N=8)	55	2.2	0.2	3.7	0.7	0.7	0.2

Body mass ( $M_b$ ), haematocrit (Hct) and blood lactate concentration are also indicated.

Within each experiment, values given are means  $\pm$  S.E.M. (N=3 blood samples at 40, 50 and 60 min of infusion).

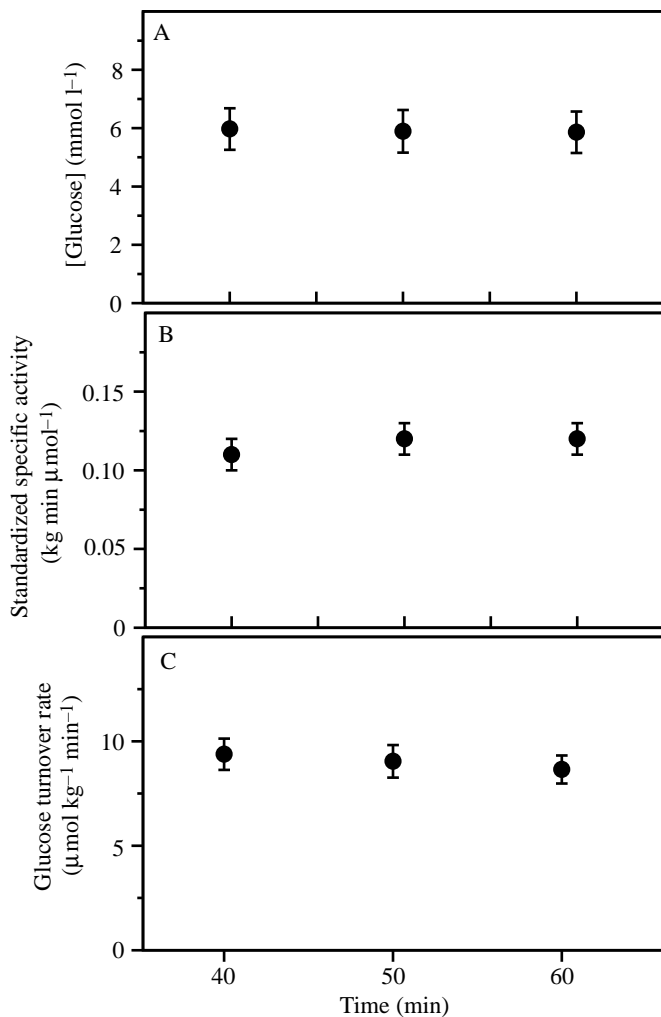


Fig. 2. Blood glucose concentration (A), standardized glucose specific activity (=specific activity/infusion rate) (B) and glucose turnover rate (C) during primed continuous infusions of 6-<sup>3</sup>H]glucose in rainbow trout. Values are means  $\pm$  S.E.M. ( $N=8$ ).

were not significantly correlated ( $r=0.49$ ,  $P=0.22$ ). Glucose clearance rate ranged between 0.9 and 2.7 ml kg<sup>-1</sup> min<sup>-1</sup> and averaged  $1.7 \pm 0.2$  ml kg<sup>-1</sup> min<sup>-1</sup>.

### Discussion

This paper describes a double dorsal aorta catheterization technique allowing the easy measurement of substrate turnover rates by continuous infusion of metabolic tracers in rainbow trout. The time necessary to carry out this new surgical procedure is only slightly longer than for a single aortic cannulation (Soivio *et al.* 1975) and it can be performed with minimal training. Moreover, the animals recover rapidly from a simple operation causing negligible blood loss and no more stress than the commonly used single cannulation. Other, less satisfactory techniques were considered before selecting the method described in this paper; the most promising alternative was to place the infusion catheter in the coeliac artery and to

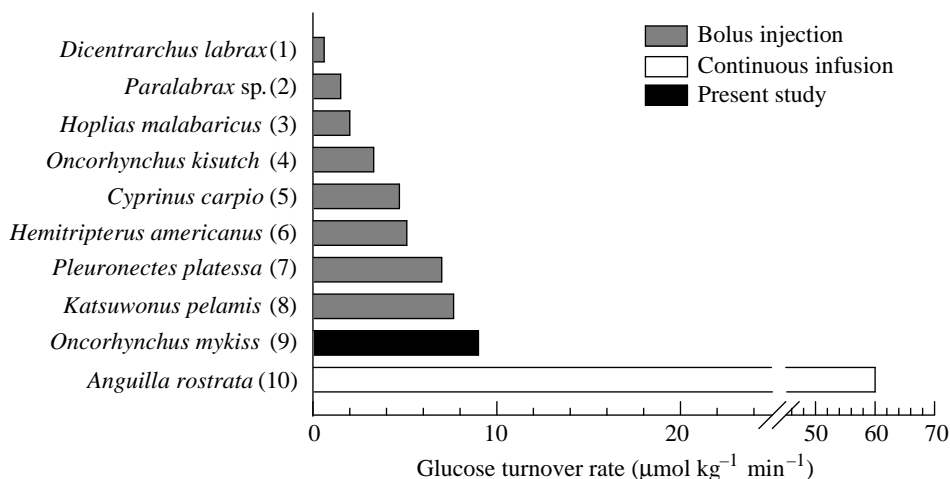
sample blood from the dorsal aorta. However, this approach was eventually abandoned because coeliac artery cannulation is far more invasive, and therefore much more stressful (i.e. longer surgery time, more blood loss and a large external wound to heal). In addition, we found it impossible to reach isotopic steady state when labelled glucose was administered through the coeliac artery, even after prolonged infusion in the healthiest animals tested.

The technique proposed in this paper makes it possible to measure metabolic substrate fluxes by continuous infusion in rainbow trout, instead of relying on the commonly used, but much less versatile, bolus injection method (Weber and Haman, 1995; Weber and Zwingelstein, 1995). There are three main reasons why using continuous infusion is advantageous for fish metabolism studies: (1) this technique has been shown to yield much more accurate estimates of substrate flux than bolus injection under steady-state conditions (when concentration and flux of the metabolite investigated are constant throughout the experiment) (Allsop *et al.* 1978, 1979); (2) bolus injection cannot be used to monitor rates of appearance and disappearance independently under non-steady-state conditions (Hetenyi *et al.* 1983); and (3) a single value of flux can be measured with much less blood (only one or two blood samples are necessary using continuous infusion, whereas a complete specific activity decay curve must be generated using bolus injection) (Wolfe, 1992).

As a practical example, we measured the glucose kinetics of resting rainbow trout to establish the necessary experimental conditions for future use of the proposed technique in this species. If the glucose pool is initially primed with the equivalent of a 90 min infusion, isotopic steady state is achieved in less than 40 min with infusion rates ranging between  $30 \times 10^3$  and  $110 \times 10^3$  disintegrations min<sup>-1</sup> kg<sup>-1</sup> min<sup>-1</sup>. For the measurement of glucose turnover rate, injecting a priming dose is necessary to reach steady state rapidly enough, because the total glucose pool of vertebrates is known to be large (reaching steady state without priming the pool would take several hours). In all our experiments, glucose concentration and specific activity remained constant after 40 min (Fig. 2), and these results show that, beyond this time, the proposed experimental set-up can be used to quantify rates of glucose production and disposal under non-steady-state conditions if required (e.g. during swimming, when water quality is altered or when hormonal manipulations are imposed on the animal).

Resting glucose turnover rates of rainbow trout averaged  $9.0 \pm 0.7$  μmol kg<sup>-1</sup> min<sup>-1</sup> (Table 1) and variability among individuals was low [ $R_t$  ranging between 6.2 and 11.8 μmol kg<sup>-1</sup> min<sup>-1</sup> ( $N=8$ )]. In addition, all our fish were in a true resting state during the infusions because they showed normal resting metabolic rates for this species and their blood lactate concentration remained low throughout the experiments. We were able to find three comparable published values of resting glucose  $R_t$  in rainbow trout, all of them measured by bolus injection at 13–15 °C (Andersen *et al.* 1991; Cowey *et al.* 1977; Washburn *et al.* 1992). These values were 4–9 times lower (ranging from 1 to 2.4 μmol kg<sup>-1</sup> min<sup>-1</sup>) than

Fig. 3. Resting glucose turnover rates measured using bolus injection or continuous infusion in 10 teleost species. Measurements made at different temperatures have been corrected to 15 °C using a  $Q_{10}$  of 2. Values are taken from (1) Garin *et al.* (1987); (2) Bever *et al.* (1977, 1981); (3) Machado *et al.* (1989); (4) Lin *et al.* (1978); (5) West *et al.* (1994a); (6) Vijayan and Moon (1994); (7) Batty and Wardle (1979); (8) Weber *et al.* (1986); (9) present study; (10) Cornish and Moon (1985).



we measured here using continuous infusion. It is difficult to compare these values with ours because the bolus injection method is much more prone to experimental errors. In the studies by Andersen *et al.* (1991) and Cowey *et al.* (1977), the first blood sample was only taken 60 min after isotope injection, and the most crucial portion of the decay curve was therefore totally missed. An accurate estimate of the surface area under the specific activity decay curve would only have been possible if the early part of the curve had been defined adequately (i.e. many samples must be taken during the first 60 min after injection to generate a usable decay curve). Furthermore, their animals were not truly at rest; they were taken repeatedly out of water for isotope injection and to sample blood from the caudal vein (Cowey *et al.* 1977), they showed hyperglycaemia (Andersen *et al.* 1991) or they were anaesthetized with MS-222 shortly before isotope injection (Washburn *et al.* 1992). Contrary to these three studies, West *et al.* (1994b) recently found resting glucose turnover rates twice as high as were measured here, but these high fluxes were probably caused by an unusually high plasma glucose concentration (averaging 20 mmol l<sup>-1</sup>). In the present study, glucose concentration and turnover rate were not correlated. In contrast, West *et al.* (1994a,b) found a significant correlation in both hyperglycaemic trout (6–38 mmol l<sup>-1</sup> plasma glucose) and carp (3–17 mmol l<sup>-1</sup>). These results suggest that flux and concentration only show a significant correlation when the animals become hyperglycaemic.

Resting glucose turnover rates have also been measured in several fish species other than trout, and Fig. 3 summarizes published results. Except for a single study where continuous infusions were carried out in American eels *Anguilla rostrata* (Cornish and Moon, 1985), glucose turnover rates were always measured using the bolus injection technique. Interestingly, the bolus injection experiments all provided lower estimates of turnover rate than we observed here for trout, whereas the American eel values previously obtained by continuous infusion were six times higher. Critical problems with the use of the bolus injection technique have been mentioned above or discussed previously (Weber and Haman, 1995). Because

continuous infusion bypasses many of these problems (Wolfe, 1992), and because it is known to be more accurate than the bolus injection technique (Allsop *et al.* 1978, 1979), previous estimates of glucose turnover measured by bolus injection probably underestimate true rates.

In conclusion, the simple surgical technique described here makes the accurate measurement of metabolite turnover rates possible in live trout and opens the door to the dynamic study of substrate kinetics under a variety of experimental conditions. The method can be adapted to the investigation of most metabolic substrates, including fatty acids, glycerol, amino acids and lactate, in addition to glucose. In particular, infusion/sampling sites may have to be modified to provide the best possible estimates of flux for metabolites entering the circulation at multiple locations. Future use of the continuous infusion technique under steady-state and non-steady-state conditions will add a new dimension to our understanding of fish metabolism.

## References

- ALLSOP, J. R., WOLFE, R. R. AND BURKE, J. F. (1978). The reliability of rates of glucose appearance *in vivo* calculated from constant tracer infusions. *Biochem. J.* **172**, 407–416.
- ALLSOP, J. R., WOLFE, R. R., DiSTEPHANO III, J. J. AND BURKE, J. F. (1979). The reliability of rates of glucose appearance *in vivo* calculated from single tracer injections. *Can. J. Physiol. Pharmacol.* **57**, 1267–1274.
- ANDERSEN, D. E., REID, S. D., MOON, T. W. AND PERRY, S. F. (1991). Metabolic effects associated with chronically elevated cortisol in rainbow trout (*Oncorhynchus mykiss*). *Can. J. Fish. Aquat. Sci.* **48**, 1811–1817.
- BATTY, R. S. AND WARDLE, C. S. (1979). Restoration of glycogen from lactic acid in the anaerobic swimming muscle of plaice *Pleuronectes platessa* L. *J. Fish Biol.* **15**, 509–519.
- BERGMEYER, H. U. (1985). *Methods of Enzymatic Analysis*. Weinheim: VCH.
- BEVER, K., CHENOWETH, M. AND DUNN, A. (1977). Glucose turnover in kelp bass (*Paralabrax* sp.): *in vivo* studies with (6-<sup>3</sup>H, 6-<sup>14</sup>C)glucose. *Am. J. Physiol.* **232**, R66–R72.

- BEVER, K., CHENOWETH, M. AND DUNN, A. (1981). Amino acid gluconeogenesis and glucose turnover in kelp bass (*Paralabrax* sp.). *Am. J. Physiol.* **240**, R246–R252.
- CORNISH, I. AND MOON, T. W. (1985). Glucose and lactate kinetics in American eel *Anguilla rostrata*. *Am. J. Physiol.* **249**, R67–R72.
- COWEY, C. B., DE LA HIGUERA, H. AND ADRON, J. W. (1977). The effect of dietary composition and of insulin on gluconeogenesis in rainbow trout (*Salmo gairdneri*). *Br. J. Nutr.* **38**, 385–395.
- GARIN, D., ROMBAUT, A. AND FRÉMINET, A. (1987). Determination of glucose turnover in sea bass *Dicentrarchus labrax*. Comparative aspects of glucose utilization. *Comp. Biochem. Physiol.* **87B**, 981–988.
- HETENYI, G. J., PEREZ, G. AND VRANIC, M. (1983). Turnover and precursor–product relationships of non-lipid metabolites. *Physiol. Rev.* **63**, 606–667.
- KATZ, J. (1979). Use of isotopes for the study of glucose metabolism *in vivo*. *Techn. metab. Res.* **B 207**, 1–22.
- KATZ, J. (1992). On the determination of turnover *in vivo* with tracers. *Am. J. Physiol.* **263**, E417–E424.
- KATZ, J., ROSTAMI, H. AND DUNN, A. (1974). Evaluation of glucose turnover, body mass and recycling with reversible and irreversible tracers. *Biochem. J.* **142**, 161–170.
- LEVY, J. C., BROWN, G., MATTHEWS, D. R. AND TURNER, R. C. (1989). Hepatic glucose output measured with labeled glucose to reduce negative errors. *Am. J. Physiol.* **257**, E531–E540.
- LIN, H., ROMSOS, D. R., TACK, P. I. AND LEVEILLÉ, G. A. (1978). Determination of glucose utilization in coho salmon (*Oncorhynchus kisutch*, Walbaum) with ( $6\text{-}^3\text{H}$ ) and ( $\text{U-}^{14}\text{C}$ ) glucose. *Comp. Biochem. Physiol.* **59A**, 189–191.
- MACHADO, C. R., GAROFALO, M. A. R., ROSELINO, J. E. S., KETTLEHUT, I. C. AND MIGLIORINI, R. H. (1989). Effect of fasting on glucose turnover in a carnivorous fish (*Hoplias* sp.). *Am. J. Physiol.* **256**, R612–R615.
- MARSH, R. L. AND DAWSON, W. R. (1982). Substrate metabolism in seasonally acclimatized American goldfinches. *Am. J. Physiol.* **242**, R563–R569.
- MOATES, J. M., LACY, D. B., GOLDSTEIN, R. E., CHERRINGTON, A. D. AND WASSERMAN, D. H. (1988). Metabolic role of the exercise-induced increment in epinephrine in the dog. *Am. J. Physiol.* **255**, E428–E436.
- MOLINA, J. M., BARON, A. D., EDELMAN, S. V., BRECHTEL, G., WALLACE, P. AND OLEFSKY, J. M. (1990). Use of variable tracer infusion method to determine glucose turnover in humans. *Am. J. Physiol.* **258**, E16–E23.
- NORWICH, K. H. (1992). Sites of infusion and sampling for measurement of rates of production in steady state. *Am. J. Physiol.* **263**, E817–E822.
- SMITH, L. S. AND BELL, G. R. (1964). A technique for prolonged blood sampling in free-swimming salmon. *J. Fish. Res. Bd Can.* **21**, 711–717.
- SOIVIO, A., NYHOLM, K. AND WESTMAN, K. (1975). A technique for repeated sampling of the blood of individual resting fish. *J. exp. Biol.* **62**, 207–217.
- STEELE, R. (1959). Influences of glucose loading and of injected insulin on hepatic glucose output. *Ann. N.Y. Acad. med. Sci.* **82**, 420–430.
- STEFFENSEN, J. F. (1989). Some errors in respirometry of aquatic breathers: how to avoid and correct for them. *Fish. Physiol. Biochem.* **6**, 49–59.
- VIJAYAN, M. M. AND MOON, T. W. (1994). The stress response and the plasma disappearance of corticosteroid and glucose in a marine teleost, the sea raven. *Can. J. Zool.* **72**, 379–386.
- WASHBURN, B. S., BRUSS, M. L., AVERY, E. H. AND FREEDLAND, R. A. (1992). Effects of estrogen on whole animal and tissue glucose use in female and male rainbow trout. *Am. J. Physiol.* **32**, R1241–R1247.
- WEBER, J.-M., BRILL, R. W. AND HOCHACHKA, P. W. (1986). Mammalian metabolite flux rates in a teleost: lactate and glucose turnover in tuna. *Am. J. Physiol.* **250**, R452–R458.
- WEBER, J.-M. AND HAMAN, F. (1995). Pathways for metabolic fuels and oxygen in high performance fish. *Comp. Biochem. Physiol.* **113A** (in press).
- WEBER, J.-M. AND ZWINGELSTEIN, G. (1995). Circulatory substrate fluxes and their regulation. In *Biochemistry and Molecular Biology of Fishes*, vol. 4, chapter 2 (ed. P. W. Hochachka and T. P. Mommsen), pp. 15–32. Amsterdam: Elsevier.
- WEST, T. G., BRAUNER, C. J. AND HOCHACHKA, P. W. (1994a). Muscle glucose utilization during sustained swimming in the carp (*Cyprinus carpio*). *Am. J. Physiol.* **267**, R1226–R1234.
- WEST, T. G., SCHULTE, P. S. AND HOCHACHKA, P. W. (1994b). Implication of hyperglycemia for post-exercise resynthesis of glycogen in trout skeletal muscle. *J. exp. Biol.* **189**, 69–84.
- WOLF, K. (1963). Physiological saline for fresh water teleosts. *Progve Fish Cult.* **25**, 135–140.
- WOLFE, R. R. (1992). *Radioactive and Stable Isotope Tracers in Biomedicine. Principles and Practice of Kinetic Analysis*. New York: Wiley-Liss.