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A STUDY OF *IN VIVO* GLYCINE ABSORPTION BY FED AND FASTED RAINBOW TROUT (*SALMO GAIRDNERI*)

BY G. BOGÉ, A. RIGAL AND G. PÉRÈS

Laboratoire Maritime de Physiologie, Institut Michel Pacha, 83500 – Tamaris sur Mer, France

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SUMMARY

The effects of 4 and 8 weeks fasting at 16 °C were studied in rainbow trout, *Salmo gairdneri* Richardson. After 4 and 8 weeks, the wet weights of the intestine of fasted animals are respectively 64% and 69% lower than those of fed animals. These effects especially concern the mucosal tissue.

Glycine absorption (0·5 and 10 mM) was studied using an *in vivo* perfusion technique. After 4 weeks, the absolute amounts of 0·5 mM glycine absorbed by fasted and fed fish are similar. With 10 mM glycine, the absorption is slightly lower in fasted trout (−19%). After 8 weeks these differences are more marked, with glycine concentrations of 10 mM (−42%). Results expressed per 100 g body weight showed that these differences result partly from a weight gain of fed trout. Absorption expressed in terms of weight of dry intestine is higher in 4 and 8 weeks fasted animals, principally for the lower amino acid concentration (+61% and +111%). Larger differences were apparent when the absorptions were expressed in terms of dry weight of mucosal tissue (+122% and +225%).

INTRODUCTION

One of the most interesting aspects of the physiology of fish is their great resistance to starvation. They are well adapted to mobilize lipids, carbohydrates, but especially proteins and amino acids, stored in the spleen, the liver, the intestine or the kidneys (Love, 1970). In the intestine, starvation causes an important loss of weight (Creach & Cournède, 1965) involving a marked atrophy when feeding has been stopped for a long period of time (Bertin, 1942). Loss of enzyme activities (Noda, 1967) and cytofunctional alterations of enterocytes (Gas, 1976; Gas & Noailliac-Depeyre, 1976) are also induced, suggesting that intestinal absorption is affected by fasting.

In order to specify the effects of fasting on the intestinal function of rainbow trout, we investigated the absorption of glycine, using an *in vivo* perfusion method. Experiments were conducted during the warm season to avoid the effects of natural fasting which occurs at low temperatures during the maturation of gonads.

MATERIALS AND METHODS

Animal maintenance

Experiments were carried out during June and July, at a water temperature of 16 ± 1 °C and with an oxygen level of about 8 mg/l. Rainbow trout (*Salmo gairdneri*) weighing about 160 g were kept in two lattice-work cages immersed in the river Gapeau (Var, France). All fish were fed for the first 3 weeks with trout pellets provided *ad libitum* 3 times a week: after that, half of them were deprived of food for a period of 4 or 8 weeks.

In vivo *technique*

The perfusion method for the rainbow trout intestine was described by Bogé, Rigal & Pérès (1977). The intestine was cannulated with flexible catheters. The unanesthetized free-swimming rainbow trout were perfused 24 h later. The perfusate had this composition (mM): NaCl 120, KCl 4·8, $CaCl_2$ 2, $MgSO_4$ 1·2 and Na_2HPO_4 15·6. The pH was 7·4. The perfusates contained either 0·5 or 10 mM glycine ^{14}C (specific radioactivity $2{\cdot}75 \times 10^4$ dis/min per ml).

The *in vivo* experiments were performed at the acclimatization temperature of the fish (i.e. 16 °C) for 30 min periods. Glycine absorption was calculated from the changes in the total radioactivity in the perfusate during each 30 min perfusion. The water content per unit of dry weight (R_D) was measured on perfused and non-perfused intestines dried at 104 °C for 24 h ($R_D = W/D$ when W is the water content and D the dry weight).

Statistical calculations

The significance of differences between the results in the different experimental groups was analysed by the Student dependent t test. A P value at the level of 0·05 or less was considered statistically significant.

RESULTS

Body weight

After 8 weeks fasting, the differences in weight between the fed and the unfed trout are highly significant (Table 1). This results largely from an increase in weight of the fed trout. These fish, whose initial weight was 160 g, gained 29 g after 4 weeks and 49 g after 8 weeks, whereas the fasting trout did not decrease in weight.

Intestinal weight

The appreciable difference in body weight between fasted and fed trout is accompanied by a drop in intestinal wet and dry weight that is particularly severe during the first four weeks (Table 2). This reduction in weight affects the different tissues of the intestine unequally: it is more impressive in the mucosa than in muscular tissue.

Because of this large reduction in gut weight, the intestinal/body weight ratio (I.B.R.) is lower in fasted trout.

The hydration of the intestine and of its mucosal layers, as indicated by the R_D values, is slightly increased, but not significantly, after 8 weeks fasting.

Table 1. *Body weight of fed and fasted trout*

	Body weight (g)	
	Fed	Fasted
After 4 weeks ($n = 15$)	189 ± 7	160 ± 6**
After 8 weeks ($n = 14$)	209 ± 7	160 ± 5**

** $P < 0.01$.
Values are means ± S.E.M., n is the number of animals used. Initial average weight 160 g.

Table 2. *Weight of wet and dry tissue, water content per unit of dry weight (R_D) of intestine, mucosal and muscular tissues and intestinal wt./body wt. ratio* (I.B.R.) *of fed and fasted (4 or 8 weeks) trout*

	After 4 weeks ($n = 8$)		After 8 weeks ($n = 6$)	
	Fed	Fasted	Fed	Fasted
Intestine				
Wet wt. (g)	2·413 ± 0·132	0·876 ± 0·048** (−64%)	2·675 ± 0·416	0·837 ± 0·042** (−69%)
Dry wt. (g)	0·498 ± 0·064	0·169 ± 0·019** (−66%)	0·556 ± 0·106	0·144 ± 0·025** (−74%)
R_D	4·24 ± 0·45	4·40 ± 0·31	4·04 ± 0·51	5·25 ± 0·56
Mucosal tissue				
Wet wt. (g)	1·593 ± 0·091	0·406 ± 0·036** (−75%)	1·697 ± 0·314	0·351 ± 0·024** (−79%)
Dry wt. (g)	0·344 ± 0·041	0·083 ± 0·010** (−76%)	0·369 ± 0·079	0·065 ± 0·011** (−82%)
R_D	3·92 ± 0·39	4·06 ± 0·27	3·81 ± 0·47	4·80 ± 0·50
Muscular tissue				
Wet wt. (g)	0·820 ± 0·051	0·470 ± 0·025** (−43%)	0·978 ± 0·106	0·486 ± 0·022** (−50%)
Dry wt. (g)	0·163 ± 0·023	0·087 ± 0·010** (−47%)	0·194 ± 0·030	0·080 ± 0·014** (−59%)
R_D	4·49 ± 0·47	4·67 ± 0·34	4·35 ± 0·58	5·59 ± 0·62
I.B.R. ($\times 10^{-2}$)	12·4 ± 0·6	5·6 ± 0·2**	12·5 ± 1·4	5·4 ± 0·2**

** $P < 0.01$.
Values are means ± S.E.M., n is the number of animals used.
The weight is determined using non-perfused trout.

Table 3. *Weight of wet and dry tissue, water content per unit of dry weight (R_D) of intestine determined at the end of* in vivo *perfusions*

	Fed	Fasted
After 4 weeks ($n = 7$)		
Wet wt. (g)	1·870 ± 0·113	1·089 ± 0·145
Dry wt. (g)	0·263 ± 0·012	0·153 ± 0·013
R_D	6·07 ± 0·12	6·05 ± 0·52
After 8 weeks ($n = 8$)		
Wet wt. (g)	2·444 ± 0·193	1·393 ± 0·164
Dry wt. (g)	0·348 ± 0·025	0·159 ± 0·090
R_D	6·08 ± 0·34	7·29 ± 0·74

Values are means ± S.E.M., n is the number of animals used.

Table 4. In vivo *study of* 0·5 *and* 10 mM *glycine absorption in fed and fasted* (4 *or* 8 *weeks*) *trout, expressed as the absolute amounts absorbed. The test perfusion lasted* 30 *min at* 16 °C

	Absolute amounts of glycine absorbed (μ mol./30 min)					
	0·5 mM glycine			10 mM glycine		
	Fed	Fasted		Fed	Fasted	
After 4 weeks ($n = 7$)	1·697 ± 0·286	1·586 ± 0·314	−7%	19·992 ± 3·569	16·175 ± 3·863	−19%
After 8 weeks ($n = 8$)	2·086 ± 0·303	2·007 ± 0·138	−4%	30·399 ± 2·863	17·648** ± 1·746	−42%

** $P < 0{\cdot}01$.

Values are means ± S.E.M. of n experiments.

Glycine absorption

Table 3 shows the intestinal weight of animals used for *in vivo* experiments. The segment perfused was slightly shorter than that included between the last pyloric caecum and the anus. It became more hydrated during perfusions, particularly on fasted intestines, as shown by the ratio of water per unit dry weight of perfused (Table 3) and non-perfused intestines (Table 2).

Glycine absorption was studied on each trout at two different concentrations of amino acid. Results were calculated according to the absolute amounts absorbed, or per weight of dry intestine. In order to take the increase in weight of fed fish into account, results were also expressed as μmoles of glycine absorbed per 100 g body weight.

1. *Absolute amounts absorbed*

Despite important differences in intestinal weight between fed and fasted trout, absolute glycine uptake is similar in both groups of animals after 4 weeks (Table 4).

After 8 weeks, differences between fasted and fed trout exist. They result in a decrease in the absorption of the highest concentration of glycine (10 mM) by the depleted animals, whereas the absolute absorption of 0·5 mM glycine does not change. These differences are emphasized by an enhanced absorption in fed trout, especially for 10 mM glycine.

2. *Amounts absorbed per* 100 *g body weight*

Expressing absorption on a body weight basis minimizes the effects of weight increases on intestinal absorption in fed trout. The increase of glycine absorption (10 mM) by these fish between 4 and 8 weeks is then no longer significant (Table 5).

Differences, recorded in Table 4, in the absorption of 10 mM glycine between fed and starved trout are also reduced.

There is a slight, but not significant, increase in the absorption of 0·5 mM glycine by fasted trout.

Table 5. In vivo *study of* 0·5 *and* 10 *mM glycine absorption in fed and fasted* (4 *or* 8 *weeks*) *trout, expressed as absorption per* 100 *g body weight. The test perfusion lasted* 30 *min at* 16 °*C*

	Absorption per 100 g body wt. (μmol/30 min)					
	0·5 mM glycine			10 mM glycine		
	Fed	Fasted		Fed	Fasted	
After 4 weeks (n = 7)	0·980 ± 0·195	0·951 ± 0·182	−3 %	11·302 ± 2·233	9·731 ± 2·349	−14 %
After 8 weeks (n = 8)	1·024 ± 0·144	1·265 ± 0·062	+20 %	14·964 ± 1·453	11·210 ± 1·151	−25 %

Values are means ± S.E.M. of n experiments.

Table 6. In vivo *study of* 0·5 *and* 10 *mM glycine absorption in fed and fasted* (4 *or* 8 *weeks*) *trout, expressed as the absorption per g dry intestine determined at the end of perfusions. The test perfusion lasted* 30 *min at* 16 °*C*

	Absorption per g dry intestine (μmol./30 min)					
	0·5 mM glycine			10 mM glycine		
	Fed	Fasted		Fed	Fasted	
After 4 weeks (n = 7)	6·595 ± 1·286	10·639* ± 2·506	+61 %	74·724 ± 11·232	101·87* ± 18·56	+36 %
After 8 weeks (n = 8)	5·968 ± 0·803	12·607** ± 0·427	+111 %	87·566 ± 7·533	111·34* ± 9·241	+27 %

** $P < 0{\cdot}01$; * $P < 0{\cdot}05$.
Values are means ± S.E.M. of n experiments.

Table 7. In vivo *study of* 0·5 *and* 10 *mM glycine absorption in fed and fasted* (4 *or* 8 *weeks*) *trout, expressed as the absorption per g dry mucosal tissue. The test perfusion lasted* 30 *min at* 16 °*C*

	Absorption per g mucosal tissue (μmol./30 min)					
	0·5 mM glycine			10 mM glycine		
	Fed	Fasted		Fed	Fasted	
After 4 weeks (n = 7)	9·838 ± 1·926	21·839* ± 5·935	+122 %	110·72 ± 15·217	224·67* ± 42·647	+103 %
After 8 weeks (n = 8)	9·661 ± 1·296	31·388** ± 1·989	+225 %	141·75 ± 12·163	276·22** ± 24·807	+95 %

** $P < 0{\cdot}01$; * $P < 0{\cdot}05$.
Values are means ± S.E.M. of n experiments.

3. *Absorption per g dry tissue*

It follows from the preceding observations that absorption expressed per gram of dry intestine is enhanced in fasted trout (Table 6) after 4 weeks and 8 weeks.

It is more interesting to assess absorption in terms of dry weight of mucosa, because of the major role played by this tissue in transport processes (Table 7). The results

expressed in this way confirm those expressed per weight of dry intestine. Nevertheless they are more striking: absorption of glycine by fasted intestines is particularly high and the increase obtained after 8 weeks fasting is more significant with 0·5 mM glycine than with 10 mM glycine.

DISCUSSION

The life history of fish frequently includes important periods of starvation. These periods occur during the production of eggs or sperm, during spawning, or during the cold season when temperatures are low (Greene, 1926; Steffens, 1964; Love, 1970; Tautz & Groot, 1975). This paper demonstrates that immature trout are also able to withstand 8 weeks starvation during a season when waters are warm enough to incite fish to set about searching for food. There was no significant loss in body weight during this period (Table 1). With carp (*Cyprinus carpio*), a similar period of fasting induces a more significant decrease of body weight, which nevertheless does not exceed 10% (Creach, 1972). With trout, however, it is possible that the starvation was not as complete as expected since the trout kept in cages immersed in the river had opportunity to catch insects or other small prey from their environment.

During experimental starvation, different organs are depleted to supply fish with substances required for the maintenance of metabolic activity (Love, 1970). In fasted trout, the intestine, which is particularly affected by food deprivation, is presumably used for this energy supply. The weight loss of this organ is very marked during the first 4 weeks and is reduced during the following weeks (Table 2). Measuring the weight loss of the intestine, kidney, liver, spleen, muscles and heart of carp, Creach & Cournède (1965) found that the intestine was the most depleted organ after 2 months starvation.

Among the principal tissues of the trout intestine, the mucosa is the most altered by fasting (Table 2). With carp, the involution of this tissue induces a diminution of the absorption surface by progressive erosion of the surface relief (Gas, 1976; Gas & Noailliac-Depeyre, 1976). With trout, histological observations on fasted intestines showed a decrease in the thickness of the mucosal epithelium.

Thus, even though there is a quantitative reduction of the absorbent tissue, the effects of prolonged starvation on absolute amounts of glycine absorbed by trout intestines are restricted. There is no difference between fed and fasted animals after 4 weeks treatment. After 8 weeks, the absorption of 0·5 mM glycine is maintained, whereas the absolute transport of 10 mM glycine by fasted trout is reduced (Table 4). This is largely due to the gain in weight of fed trout over a period of 8 weeks, because there are fewer differences when results are related to 100 g body weight (Table 5).

In order to take into account the effects of the weight loss of intestinal tissue during fasting on absorption, results are generally referred to as wet or dry weight. We have reservations about expressing the *in vivo* results in terms of wet weight, since the water content of the intestines changes during the experiments: an increase in water content per unit of dry weight (R_D) was found in perfused intestine, especially after fasting (Table 3). This hydration does not alter the glycine transport, since we ensured that the amounts of amino acid transported during succeeding perfusions did not change. However, it would dilute out differences between fed and fasting trout. This is why we considered the dry weight of intestine to be a more satisfactory

reference for comparing glycine absorption. Another reason for expressing the absorption on the basis of g dry intestine is that absorption is then unchanged in fed trout after 4 and 8 weeks. It then becomes possible to discuss the effects of fasting independently of the intake in weight of fed trout. For example, it can be shown that fasting results in an enhanced absorption in fasted intestines (Table 6). However, the values per g intestine can be misconstrued when the mucosal tissue is more depleted than the non-absorbing structures (Henaghan, 1963; Levin, 1970). Therefore, absorption expressed in terms of mucosal dry weight seems more representative of the intestinal transport capacities during fasting. It would be more satisfactory to assess absorption on the basis of mucosal surface area. Unfortunately its measurement, according to the method proposed by Bergot, Solari & Luquet (1975) was not as convenient as the measurement of the dry weight of the mucosal tissue. By using this measurement, the increase of the glycine transport observed on the basis of g intestine is corroborated, especially after 8 weeks fasting with the lowest concentration of glycine (0·5 mM in Table 7).

These results are similar to those of Levin (1970) dealing with the effects of 3 days starvation on glycine transport by rat intestines. In spite of a loss of 36% of intestinal weight, the amounts of glycine (5 mM) absorbed were not reduced in fasted intestines. On the other hand, at a concentration of 30 mM, there was a decrease in the absolute absorption of glycine. Moreover, absorption expressed per g wet intestine is enhanced in starved rats, principally for the lowest concentration. With rat, starvation results in an increase in the intestinal transport of amino acid, through combined effects on both active and passive pathways (Newey, Sanford & Smyth, 1970; Yasumoto, Sugiyama & Mitsuda, 1977). With trout, in the absence of information concerning the effects of fasting on mesenteric blood and lymph flow, on hormone secretion and on gut motility, it can be put forward that fasting can induce an increase in the intestinal permeability. This in turn limits the decrease of absolute absorption caused by the loss of a great proportion of mucosal tissue.

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