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Effect of the Pgm1-t regulatory gene on liver metabolism in rainbow trout

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EFFECT OF THE Pgm1-t REGULATORY GENE ON LIVER METABOLISM IN RAINBOW TROUT

By

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ABSTRACT

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The effects of the regulatory gene Pgm1-t on liver metabolism were investigated in rainbow trout. The Pgm1-t locus regulates the expression of a phosphoglucomutase locus (Pgm1) in the liver. Liver glycogen, glucose-6-phosphate (G6P) levels, hepatosomatic index (HSI), condition factor (K), glycogen phosphorylase activity, and glycogen synthase activity are reported for fish with and without PGM1 liver expression. Data were recorded for fish fed a commercial hatchery diet, a high-carbohydrate (HC) diet, and during starvation.

PGM1 expression in the liver has a significant effect on glycogen metabolism. Glycogen levels, HSI, and G6P levels in the liver support the conclusion that fish with PGM1 liver expression have greater flux through the pathways of glycogen storage and mobilization than fish lacking PGM1 liver expression. G6P levels in the liver are significantly lower during normal and HC feeding in fish with PGM1 liver expression than in fish without PGM1 liver expression. G6P levels in the liver are significantly higher during starvation in fish with PGM1 liver expression than in fish lacking PGM1 liver expression. Fish with PGM1 expression in the liver tend to store higher levels of glycogen during normal and HC feeding than fish lacking PGM1 liver expression. Results from HSI, a sensitive indicator of liver glycogen levels, also support the above conclusion. Fish with PGM1 expression in the liver have a faster gain in HSI during HC feeding and a faster decline in HSI during starvation than fish lacking PGM1 liver expression.

PGM1 expression in the liver has a significant effect on homeostasis. Fish with PGM1 liver expression exhibit greater homeostasis in the liver and total body than fish lacking PGM1 liver expression. Fish with PGM1 liver expression have a lower coefficient of variation (CV) for HSI and K than fish lacking PGM1 liver expression.

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INTRODUCTION

The discovery of significant levels of genetic variation at enzyme loci is a fairly recent development. A survey of Shaw (1965) reviewed 16 different enzymes in some 20 species of organisms, from flagellates to mammals, that had shown variation in electrophoretic mobility. Lewontin (1974) summarizes much of this initial work. Research in the past 15 years has revealed a large amount of genetic variation in most species of plants and animals. Nevo et al. (1984) summarizes work on over 1000 species.

As Miller et al. (1975) point out, what we would really like to know is not how much genetic variation exists in nature but how much of the observable enzyme variation is adaptive and what proportion represents what Kimura and Ohta (1971) call "neutral" polymorphism. In order to assess the adaptive significance of an enzyme polymorphism we need to understand the affect of the allelic variants on the physiology of the organism.

In general, there is a lack of physiological correlates for enzyme variation. Most of the attempts to demonstrate the adaptive nature of allozymes have relied upon "in vitro" biochemical techniques to detect differences between allelic forms of the enzymes. It is generally assumed that any differences in the kinetic properties of the allelic variants found with "in vitro" tests will be important "in vivo". As Miller et al. (1975) suggested, however, "in vitro" studies on enzymes are always questionable because they produce values representing

nonphysiological conditions. In most cases, we do not know the actual intracellular enzyme or substrate concentrations. The properties of an enzyme are physiologically important only to the extent that they affect the flux and concentrations of intermediates in its metabolic pathway (Kacser and Burns, 1981). There are few examples where research demonstrates important physiological differences caused by enzyme polymorphism at structural loci. Leigh-Brown (1977) has shown differences in liver glycogen metabolism between field mice, Apodemus sylvaticus, having different electromorphs. Koehn et al. (1983) reviews much of the recent literature on the adaptive significance of enzyme polymorphism. There has been much activity in this area of research , however, there are very few well documented examples of the adaptive importance of enzyme polymorphisms. Some of the most important recent work has been done by Powers et al. (1983) and DiMichele and Powers (1984a, b). Their work with the LDH-B locus of Fundulus heteroclitus may be the most convincing case for the adaptive significance of an enzyme polymorphism.

The importance of regulatory genes in adaptive evolutionary change has received much attention in recent years. Regulatory genes may bring about large organismal effects by altering the rate and timing of developmental processes (Britten and Davidson, 1969; King and Wilson, 1975; Wilson, 1976). Frazetta (1970) and Gould (1980) suggested that significant changes in life-history characteristics and morphology can occur during early development from alterations caused by regulatory genes. The regulation of enzyme loci may be of greater adaptive importance than structural differences in the enzymes coded by these loci (Wallace, 1963; Wilson, 1976; Ayala and McDonald, 1981). Research on

prokaryotes suggest that adaptation to new environments often involves changes in the regulation of structural loci rather than changes in the enzymes themselves (Lin et al. 1976). Evidence for the adaptive importance of gene regulation in eukaryotes is accumulating and is reviewed in MacIntyre (1982).

Allendorf et al. (1982) reported a gene regulating the tissue expression of a phosphoglucomutase (PGM; EC 2.7.5.1) locus in rainbow trout. The gene regulates tissue-specific expression at a single structural locus, Pgm1. Fish that are homozygous at the regulatory locus, Pgm1-t(a/a), have no detectable PGM1 activity in liver tissue. Individuals with one copy of the b allele, Pgm1-t(a/b), show a greater than 100-fold increase in the amount of PGM1 enzyme in the liver (Allendorf et al. 1982). There are no apparent differences between these types in the expression of PGM1 in other tissues (skeletal muscle, eye, heart, brain, stomach, and pyloric cecum).

The results of inheritance experiments are consistent with a single regulatory gene, Pgm1-t, with additive inheritance being responsible for the large differences in expression of the Pgm1 locus in liver tissue. For convenience, fish with PGM1 expression in the liver will be called type B fish and fish lacking PGM1 liver expression will be called type A fish.

The Pgm1-t regulatory gene has significant influence on body size, developmental rate, developmental stability, meristics, and age at first reproduction (Allendorf et al. 1983a; Allendorf et al. 1983b; Leary et al. 1984). Fish with PGM1 expression in the liver are longer, on the average, than fish without PGM1 liver expression. No differenc -

es in growth rate between the Pgm1-t genotypes have been detected. Fish with PGM1 expression in the liver apparently achieve a greater size early in development and maintain this advantage. These fish have a more rapid developmental rate in the period between organogenesis (15 days) and hatching (33 days). Fish with PGM1 liver expression are less asymmetrical for bilateral meristic traits and are, therefore, considered to be more developmentally buffered than fish lacking PGM1 liver expression. The shorter developmental period for type B fish also results in lower meristic counts than those found in type A fish. Fish with PGM1 expression in the liver tend to mature earlier than fish without PGM1 liver expression.

General support for the adaptive importance of regulatory genes, such as Pgm1-t, comes from their potential affects on metabolic pathways. Electrophoretic variants are expected to have "small or negligible effects" because the total flux through a metabolic pathway is largely insensitive to even large changes at individual loci (Kacser and Burns, 1981). Thus even a 50% reduction in enzyme activity in heterozygotes for enzymatically inactive alleles is not expected to be detectable in the phenotype. The regulatory mutant at the Pgm1-t locus does not have this shortcoming. The common rainbow trout phenotype has no detectable PGM1 activity in liver tissue. Type B fish, carrying the mutant allele Pgm1-t(b), have a more than 100-fold increase in the amount of PGM1 in the liver.

The PGM enzyme has a dual role functioning in the catabolic pathway of glycogen degradation and the anabolic pathway of glycogen synthesis. PGM catalyses the reversible reaction which converts glu-

cose-1-phosphate (G1P) to glucose-6-phosphate (G6P). G6P, a substrate and product of the reversible reaction catalysed by PGM, is the only substrate common to all the pathways of glucose metabolism (Walton and Cowey, 1982). It is therefore not unexpected that a large increase in PGM activity, due to the Pgm1-t(b) allele, could have a significant effect on carbohydrate metabolism.

PGM1 expression in the liver apparently affects the flux through the pathways of glycogen metabolism. This is the probable physiological or biochemical basis for the phenotypic effects of PGM1 expression. All of these phenotypic effects are, essentially, related to developmental rate. Indirect evidence supporting the above hypothesis comes from data on the developmental rate of fish with PGM1 expression in the liver. Embryos with liver PGM1 enzyme hatch earlier than embryos without liver PGM1 (Allendorf et al. 1983a). Rainbow trout embryos generate energy by glycolysis, using glycogen as a major source of energy (Terner, 1968; Boulekbache, 1981). Glycogen is synthesized during development of the trout embryo and is present exclusively in the liver (Blaxter, 1969). The presence of PGM1 in the liver may affect the flux thru the glycolytic pathway resulting in an accelerated rate of development. Additional support for this idea is found when developmental rates are examined at higher temperatures between type A and type B fish. Glycolysis should be of greater metabolic importance at higher temperatures, when there is less oxygen available and anaerobic metabolism becomes more important (Hochachka, 1969). Therefore, you would predict the differences in developmental rates between fish with and without liver PGM1 should increase with temperature. Allendorf et al. (1983a) found

greater differences in developmental rate at higher temperatures than at lower temperatures between fish with and without PGM1 liver expression.

The greater flux through the pathways of glycogen metabolism in fish with PGM1 liver expression apparently affects developmental stability. Allendorf et al. (1983) used counts of five bilateral meristic traits to measure developmental stability. Fish with liver PGM1 activity were asymmetrical at a significantly smaller mean number of traits than fish lacking liver PGM1. This suggests that fish with liver PGM1 expression are more buffered against environmental change during development than fish without PGM1 liver expression. There should be detectable differences in the physiology of liver tissue between fish with and without PGM1 liver expression that would affect homeostasis.

Previous work on the Pgm1-t polymorphism has concentrated on exploring the phenotypic effects of PGM1 expression. The goal of this study was to test for biochemically detectable physiological effects of Pgm1-t "in vivo".

METHODS

Starvation Experiment

Fish for all experiments are from the Arlee strain (see Leary et al. 1983 for the history of this strain). Fish from family I17, segregating 1:1 (a/a : a/b) at Pgm1-t were used in this experiment. All fish were full sibs and one year of age. Fish were scored for PGM phenotype by horizontal electrophoresis on starch gels following previously described methods (Utter et al. 1974) using stains and buffer systems in Allendorf et al. (1977).

Fish were divided into two holding tanks at different temperatures. One tank was kept at 9°C and the other tank at 13°C. Twenty fish were sampled from each tank upon the start of starvation and for five successive weeks. Fish were fed Silver Cup, a commercial trout diet, prior to starvation.

Routine measurements included fork length (FL), liver weight (LW), and body weight (BW). Hepatosomatic index (HSI = LW / BW x 100) and condition factor ($K = BW / FL^3 \times 10^5$) were calculated for all fish. Liver samples were frozen upon dissection using spray freon and kept frozen until analysis. Liver glycogen was analysed using the anthrone method of Seifter et al. (1950) with the modification of Van Handel (1965).

The hepatosomatic index and condition factor are commonly used because they are convenient indices of the relative physiological state of the organism. The validity of the hepatosomatic index and condition factor were examined by regression techniques and analysis of covariance (ANCOVA). The HSI is based on the relationship $LW = aBW^b$. For HSI to

be valid, b must equal unity so that the LW/BW ratio is constant over all body weights. ANCOVA, on log-transformed data, demonstrated no significant difference in slopes between type A and B fish and no significant difference from unity. The condition factor is based on the relationship $BW = aFL^b$ where b is assumed to equal three. ANCOVA, on log-transformed data, shows homogenous slopes that do not deviate significantly from the value of three for all fish. Therefore, for purposes of convenience both HSI and K will be used in the results of this work. Research by Denton and Yousef (1976) on rainbow trout during the same period of growth agree with the above conclusions concerning the LW/BW relationships.

High-Carbohydrate Diet Experiment

Fish from families J26 and J27 segregating 1:1 (a/a : a/b) at Pgm1-t were used in this experiment. All fish were full sibs and six months old. Fish were reared and sampled at the Jocko River State Trout Hatchery. Water temperature was a constant 9 C during the experiment. Fish were scored for PGM phenotype as previously described.

Fish were fed Silver Cup trout food until the start of high-carbohydrate (HC) feeding. The HC diet was made from the formula of Hilton (1982 and personal communication). This diet contained 17% digestible carbohydrate (Table 1). This food was pelleted by Dr. Ron Hardy at the Northwest and Alaska Fisheries Center.

I sampled fish before the start of HC feeding. Fish were sampled weekly during two weeks of HC feeding. After the HC feeding period fish were starved for one week. Sampling occurred every 12 hours

Table 1. Percentage composition of the high-carbohydrate diet.

Ingredient	Percent Composition
Capelin meal	40
Soybean meal	25
Wheat middlings	15
Vitamin mix	2
Mineral mix	1
Cerelose	17

for the first two days of starvation and daily for the rest of this week. Forty fish were collected from each family at every sampling time.

Routine measurements and liver sampling were done as described earlier. Liver glycogen and G6P levels were analysed using the spectrophotometric technique of Keppler and Decker (1977).

Enzyme Activity Experiment

A special sample of fish was collected from the HC experiment. Fish were sampled before HC feeding and after two weeks of HC feeding. Tissue samples were rapidly removed and homogenized in 19 parts (w/w) of ice cold buffer. The buffer contained 40 mM Imidazole, 1.5 mM EDTA, 3 mM $MgCl_2$, 0.15 M NaF; pH 6.8. NaF was included to inhibit phosphorylase phosphatase and protein kinase activities (Stalmans and Hers, 1975). The samples were centrifuged and the supernatant used in the assays.

PGM activity was measured spectrophotometrically using a coupled assay. The assay mixture contained 40 mM Imidazole, 1.5 mM EDTA, 3 mM $MgCl_2$, 3.5 mM G1P, 0.02 mM G-1,6-P_2, 6 mM NADP, glucose-6-phosphate dehydrogenase (G6PDH, 1U / ml); pH 6.8. PGM activity was assayed in liver tissue before HC feeding and after two weeks HC feeding.

Glycogen phosphorylase activity was measured spectrophotometrically using a coupled assay. For measurement of total phosphorylase activity (PHOS a + b) the assay contained 40 mM Imidazole, 1.5 mM EDTA, 3 mM $MgCl_2$, 40 mM $NaPO_4$, 2 mM AMP, 6 mM NADP, 0.02 mM G-1,6-P_2, glycogen (2 mg / ml), PGM (1U / ml), G6PDH (1U / ml); pH 6.8. Glycogen was made

AMP free by using Dowex 1x8 (Cl^- form) ion exchange resin (Childress and Sacktor, 1970). For the measurement of phosphorylase a (PHOS a) activity AMP was omitted and 0.5 mM caffeine was added to the assay mixture (Stalmans and Hers, 1975).

Glycogen synthase activity was measured using the one-step spectrophotometric assay of Passoneau and Rottenberg (1973).

All of the above assays were run at 25°C. Results are expressed as IU / g of fresh tissue.

RESULTS

Enzyme Activities

Enzyme activity levels of phosphorylase and synthase were assayed because they are known to be important in the regulation of glycogen storage and mobilization (Cohen, 1980). I was interested in comparing the activity levels of these enzymes in type A and B fish. PGM activity levels in the liver were taken as controls. Glycogen phosphorylase and synthase activity levels were not significantly different between type A and B fish during normal feeding (Table 2). No significant change in these levels occurred after two weeks HC feeding (Table 3).

Starvation Experiment

Starvation is known to cause reduction of liver glycogen in rainbow trout (Hickling and March, 1981; Hilton, 1982). Water temperature may also affect the levels of liver glycogen. Higher water temperatures increase the rate of glycogen breakdown in trout liver (Hilton, 1982).

In this experiment, starvation at two different temperatures was used to study the effects of the Pgm1-t polymorphism on liver metabolism. I suspected that the presence of PGM1 in the liver might cause an increase in glycogen storage during normal feeding conditions and a faster depletion of glycogen stores during starvation.

Liver Glycogen

Mean glycogen levels were greater in type B fish than type A fish during normal feeding (Table 4). These differences, however, are

Table 2. Phosphoglucomutase, phosphorylase, and synthase activity in liver tissue from fish fed a normal diet. Results are expressed as IU g^{-1}(wet weight of tissue).

	Activity		
Enzyme	A	B	Probability*
PGM	40.84 ± 1.399	66.90 ± 3.631	$p < 0.001$
PHOS A + B	0.33 ± 0.032	0.35 ± 0.046	NS
PHOS A	0.26 ± 0.031	0.31 ± 0.055	NS
SYN I + D	0.25 ± 0.019	0.29 ± 0.020	NS
SYN I	0.27 ± 0.015	0.31 ± 0.017	NS

*Mann-Whitney U-Test

Table 3. Phosphoglucomutase, phosphorylase, and synthase activity in liver tissue from fish fed the high-carbohydrate diet. Results are expressed as IU g^{-1}(wet weight of tissue).

Enzyme	Activity A	Activity B	Probability*
PGM	37.77 ± 3.120	53.98 ± 2.433	P <0.001
PHOS A	0.30 ± 0.035	0.26 ± 0.030	NS
SYN I	0.40 ± 0.079	0.38 ± 0.067	NS

* Mann-Whitney U-Test

Table 4. Liver glycogen content for Family I17 during the starvation experiment. All results are expressed as uM glucosyl units g^{-1} (wet weight of tissue).

	Liver glycogen (mean ± SEM)			
	9°C		13°C	
Starvation (weeks)	A	B	A	B
0*	141 ± 15.0	201 ± 47.4		
1	76 ± 10.5	88 ± 18.5	91 ± 16.1	73 ± 14.2
2	131 ± 13.6	104 ± 17.9	122 ± 13.0	114 ± 10.5
3	119 ± 19.1	133 ± 11.7	104 ± 13.0	88 ± 9.9
4	86 ± 10.5	79 ± 5.6	61 ± 5.6	71 ± 6.8
5	68 ± 5.6	95 ± 18.5	55 ± 3.7	68 ± 8.6

*Before starvation; normal diet.

not statistically significant.

There were no significant differences in the rates of glycogen depletion between type A and B fish during starvation. Analysis of variance (ANOVA) on glycogen levels during starvation does not show a significant interaction effect between genotype and starvation. The experimental treatment (starvation) caused a significant effect ($P < .001$) upon liver glycogen levels. Liver glycogen levels declined in all fish during starvation.

Hepatosomatic Index

Liver weights are often used as indicators of physiological response to diet and to toxicants. The liver is very sensitive to changes in dietary carbohydrate. Glycogen can account for as much as 12% of total liver weight (Hilton, 1982). HSI is, therefore, a very useful index for monitoring changes in liver glycogen content.

The mean HSI was greater in type B fish than type A fish during normal feeding (B = 1.39, A = 1.30; $P < .001$, Mann-Whitney U-Test; see Table 5). ANOVA on HSI demonstrated a significant interaction effect ($p < .001$) between genotype and starvation. Examination of the data, using least significant difference (Snedecor and Cochran, 1967; abbreviated LSD) showed that type B fish had a dignificantly faster decline in mean HSI than type A fish during starvation (Table 5). At 13°C type B fish showed a significantly lower mean HSI after one week of starvation compared to two weeks for type A fish. At 9°C type B fish showed a significantly lower mean HSI after one week of starvation compared to three weeks for type A fish. Fish held at 13°C showed a faster decline in mean HSI than fish held at 9°C.

Table 5. Hepatosomatic index for Family I17 during the starvation experiment.

Starvation (weeks)	HSI (mean ± SEM) 9°C A	9°C B	13°C A	13°C B
0*	1.30 ± 0.051	1.39 ± 0.046		
1	1.30 ± 0.075	1.10 ± 0.068	1.20 ± 0.157	1.09 ± 0.049
2	1.19 ± 0.065	0.97 ± 0.058	0.97 ± 0.078	0.96 ± 0.060
3	0.98 ± 0.064	1.07 ± 0.036	0.91 ± 0.054	0.81 ± 0.043
4	1.06 ± 0.067	1.02 ± 0.053	0.79 ± 0.074	0.92 ± 0.036
5	0.90 ± 0.037	0.90 ± 0.036	0.84 ± 0.016	0.73 ± 0.053

*Before starvation; normal diet.

Condition Factor

Condition factor is frequently used to compare the relative physical condition of fish of the same species. Fish with greater K values are heavier at a given length than fish with lower K values.

There were no significant differences in the rate of decline of K between type A and B fish during starvation. Analysis of K during starvation with ANOVA showed no significant interaction effect between genotype and starvation (Table 6). Temperature had no significant effect on K during starvation. Starvation caused a significant effect on K ($P<.001$). The mean K declined for all fish during starvation.

High-Carbohydrate Diet Experiment

Diets high in digestible carbohydrate are known to result in increased liver glycogen content and increased liver weight in rainbow trout (Hilton, 1982). Glycogen utilization in trout reared on diets high in digestible carbohydrate is probably caused by reduced gluconeogenesis from precursors other than glycogen. I used high levels of digestible carbohydrate to examine the effects of the Pgm1-t polymorphism on glycogen metabolism. It might be expected that PGM1 expression in the liver would cause increased glycogen storage because of its potential influence on the rate of flux thru the pathway between G6P and glycogen. PGM1 expression in the liver should also, therefore, bring about a faster decline in liver glycogen levels during starvation.

Table 6. Condition factor for Family I17 during the starvation experiment.

	K (mean ± SEM)			
	9°C		13°C	
Starvation (weeks)	A	B	A	B
0*	1.30 ± 0.020	1.32 ± 0.016		
1	1.18 ± 0.019	1.15 ± 0.028	1.17 ± 0.018	1.14 ± 0.013
2	1.07 ± 0.018	1.08 ± 0.024	1.08 ± 0.017	1.11 ± 0.014
3	1.09 ± 0.017	1.10 ± 0.012	1.07 ± 0.009	1.10 ± 0.016
4	1.05 ± 0.018	1.06 ± 0.013	1.07 ± 0.023	1.03 ± 0.009
5	1.05 ± 0.019	1.05 ± 0.013	1.04 ± 0.021	1.03 ± 0.014

*Before starvation; normal diet.

Glucose-6-phosphate Levels

If PGM1 is having a physiological effect, then it must be affecting flux in the metabolic pathways. This can be tested by measuring in vivo substrate pools. I decided to analyse G6P levels since G6P is a substrate of PGM. Mean G6P levels are significantly lower for type B fish than type A fish before HC feeding ($P<.001$, Mann-Whitney U-Test) and after one week HC feeding ($P<.05$, Mann-Whitney U-Test). The mean G6P content is lower for type B fish than type A fish after two weeks HC feeding but is not significantly lower (Table 7). Examination of the mean G6P levels in the liver during starvation show that type B fish have a significantly greater mean level ($P<.001$, Mann-Whitney U-Test) than type A fish (Table 7).

Liver Glycogen

Mean glycogen levels are greater in the livers of type B fish than type A fish after HC feeding (Table 8). Type B fish show a decline in mean glycogen levels in the liver after 12 hours of starvation. Glycogen levels did not decrease after 12 hours of starvation in type A fish (Table 8). Analysis of glycogen levels with ANOVA, however, does not show any statistically significant effects caused by genotype during HC feeding or starvation.

Hepatosomatic Index

Type B fish for family J26 had a faster increase in HSI during HC feeding than type A fish. ANOVA of HSI for family J26 shows a marginally significant interaction effect ($P<.079$) between genotype and HC feeding. Examination of the weekly samples with LSD shows that for type A fish there are no significant differences between the mean HSI

Table 7. Glucose-6-phosphate concentration in liver tissue of Family J26 during the high-carbohydrate experiment. Results are expressed as uM g^{-1} (wet weight of tissue).

	G6P Concentration		
Diet	A	B	Probability *
Normal diet	0.32 ± 0.058	0.26 ± 0.046	$P < 0.001$
1 week HC	0.22 ± 0.056	0.09 ± 0.012	$P < 0.05$
2 weeks HC	0.26 ± 0.071	0.22 ± 0.030	NS
Starvation	0.28 ± 0.002	0.29 ± 0.003	$P < 0.001$

*Mann-Whitney U-Test

Table 8. Liver glycogen content for Family J26 during the high-carbohydrate experiment. All results are expressed as uM glucosyl units g^{-1} (wet weight of tissue).

	Liver glycogen (mean ± SEM)		
	A	B	Probability*
Normal diet	136 ± 12.8	119 ± 9.7	0.32
1 week HC	725 ± 47.9	762 ± 55.3	0.56
2 weeks HC	510 ± 49.1	566 ± 50.0	0.47
Starvation (hours)			
12	526 ± 59.1	486 ± 60.9	0.40
24	302 ± 32.4	414 ± 49.9	0.12
36	306 ± 39.7	317 ± 34.5	0.64
48	234 ± 47.3	279 ± 47.2	0.42
72	150 ± 31.8	171 ± 35.8	0.68
96	206 ± 45.9	302 ± 98.0	0.50
120	211 ± 54.9	192 ± 32.9	0.85
144	122 ± 28.7	178 ± 80.2	0.27
168	88 ± 15.0	143 ± 40.0	0.47

*Mann-Whitney U-Test

during HC feeding (before HC feeding, after one week HC or two weeks). Examination of the data for type B fish show significant differences ($P = .05$, LSD) in HSI between all samples during HC feeding (Table 9). Comparisons of the mean HSI between type A and B fish show no significant differences before HC feeding or after one week HC feeding. After two weeks HC feeding type B fish have a significantly greater mean HSI ($P<.01$, Mann-Whitney U-Test) than type A fish.

ANOVA of HSI for family J27 during HC feeding does not show a significant interaction effect between genotype and HC feeding. There are no significant differences between the means of type A and B fish before HC feeding or after one week HC feeding. After two weeks HC feeding the median HSI is significantly greater for type B fish than type A fish ($P<.04$, Mann-Whitney U-Test; see Table 10).

Examination of the means for HSI, considering data from all the above experiments (Starvation and HC Diet), show that type B fish tend to have a greater mean HSI than type A fish. In nine of eleven comparisons, during normal and HC feeding, fish with liver PGM1 expression have a greater mean HSI than full sibs lacking PGM1 expression (sign test, $X^2 = 4.45$, $P<.05$). There are four significant differences in the pairwise comparisons, and in each case, the individuals with PGM1 expression in the liver have the greater mean.

Examination of the mean HSI during starvation in families J26 and J27 show that type B fish have a faster decline in mean HSI than type A fish. ANOVA for HSI in family J26 shows a significant interaction effect ($P = .06$) between genotype and starvation. Examination of the means with LSD shows that type B fish have a significantly lower

Table 9. Hepatosomatic index for Family J26 during the high-carbohydrate experiment.

	HSI (mean ± SEM)		
	A	B	Probability*
Normal diet	1.71 ± 0.089	1.61 ± 0.098	0.24
1 week HC	1.65 ± 0.099	1.86 ± 0.098	0.12
2 weeks HC	1.84 ± 0.074	2.12 ± 0.068	0.007
Starvation (hours)			
12	2.02 ± 0.139	2.35 ± 0.150	0.04
24	2.03 ± 0.083	2.16 ± 0.081	0.35
36	1.77 ± 0.082	1.77 ± 0.094	0.85
48	1.89 ± 0.093	1.96 ± 0.073	0.34
72	1.52 ± 0.069	1.64 ± 0.065	0.40
96	1.83 ± 0.102	1.88 ± 0.136	0.65
120	1.89 ± 0.178	1.63 ± 0.062	0.51
144	1.54 ± 0.052	1.70 ± 0.139	0.33
168	1.47 ± 0.044	1.51 ± 0.055	0.51

*Mann-Whitney U-Test

Table 10. Hepatosomatic index for Family J27 during the high-carbohydrate experiment.

	HSI (mean ± SEM)		
	A	B	Probability*
Normal diet	1.44 ± 0.071	1.43 ± 0.063	0.92
1 week HC	1.94 ± 0.115	1.96 ± 0.085	0.65
2 weeks HC	1.74 ± 0.104	2.00 ± 0.097	0.04
Starvation (hours)			
12	1.56 ± 0.083	1.70 ± 0.074	0.31
24	1.36 ± 0.068	1.48 ± 0.086	0.46
36	1.38 ± 0.136	1.58 ± 0.055	0.05
48	1.52 ± 0.079	1.65 ± 0.052	0.33
72	1.49 ± 0.075	1.46 ± 0.082	0.88
96	1.62 ± 0.089	1.48 ± 0.048	0.22
120	1.38 ± 0.057	1.54 ± 0.060	0.03
144	1.58 ± 0.052	1.42 ± 0.065	0.02
168	1.36 ± 0.049	1.42 ± 0.070	0.55

*Mann-Whitney U-Test

HSI sooner after the start of starvation than type A fish (after 36 hours for type B fish compared to 72 hours for type A fish; see Table 10). At the start of starvation (after two weeks HC feeding) type B fish had a significantly greater mean HSI than type A fish (discussed above).

ANOVA for HSI in family J27 shows a significant interaction effect ($P = .06$) between genotype and starvation. Examination of the means with LSD shows that in type B fish HSI declines faster during starvation than in type A fish (significant differences after 12 hours in type B fish, 24 hours in type A fish; see Table 10).

Condition Factor

K for type A and B fish from family J26 does not differ during HC feeding or starvation. ANOVA of K for family J26 showed no significant interaction effect between genotype during HC feeding or starvation (Table 11).

Type B fish in family J27 had a faster gain in K during HC feeding than type A fish. ANOVA of K for family J27 show a significant interaction effect between genotype and HC feeding. After two weeks HC feeding type B fish had a significantly greater mean K ($P < .01$, Mann-Whitney U-Test) than type A fish (Table 12). ANOVA of K during starvation showed no significant interaction effect between genotype and starvation (Table 12).

Data for K were examined from all experiments (Starvation and HC Diet). Means for K are greater for type B fish than type A fish in a significant proportion of comparisons (9 / 11; sign test, $\chi^2 = 4.45$,

Table 11. Condition factor for Family J26 during the high-carbohydrate experiment.

	K (mean ± SEM)		
	A	B	Probability*
Normal diet	1.22 ± 0.024	1.23 ± 0.029	0.73
1 week HC	1.30 ± 0.026	1.34 ± 0.017	0.37
2 weeks HC	1.29 ± 0.034	1.30 ± 0.032	0.83
Starvation (hours)			
12	1.23 ± 0.029	1.24 ± 0.019	0.94
24	1.19 ± 0.023	1.23 ± 0.019	0.22
36	1.27 ± 0.018	1.23 ± 0.028	0.21
48	1.18 ± 0.014	1.21 ± 0.025	0.21
72	1.24 ± 0.061	1.26 ± 0.027	0.07
96	1.20 ± 0.019	1.16 ± 0.022	0.02
120	1.12 ± 0.040	1.13 ± 0.021	0.65
144	1.23 ± 0.015	1.20 ± 0.020	0.36
168	1.27 ± 0.018	1.24 ± 0.032	0.14

*Mann-Whitney U-Test

Table 12. Condition factor for Family J27 during the high-carbohydrate experiment.

	K (mean ± SEM)		
	A	B	Probability*
Normal diet	1.49 ± 0.018	1.47 ± 0.018	0.51
1 week HC	1.32 ± 0.013	1.34 ± 0.011	0.17
2 weeks HC	1.30 ± 0.013	1.37 ± 0.016	0.007
Starvation (hours)			
12	1.40 ± 0.017	1.39 ± 0.023	0.63
24	1.34 ± 0.017	1.38 ± 0.017	0.15
36	1.34 ± 0.013	1.34 ± 0.024	0.57
48	1.31 ± 0.012	1.34 ± 0.013	0.12
72	1.32 ± 0.015	1.37 ± 0.049	0.46
96	1.22 ± 0.016	1.23 ± 0.015	0.59
120	1.22 ± 0.014	1.23 ± 0.019	0.43
144	1.23 ± 0.013	1.23 ± 0.016	0.87
168	1.27 ± 0.019	1.27 ± 0.019	0.47

*Mann-Whitney U-Test

$P < .05$) for fish fed a normal hatchery diet or HC diet. There was one significant pairwise difference with type B fish having the greater mean than type A fish.

Homeostasis

Homeostasis refers to the ability of an organism to regulate or stabilize ifself in the face of fluctuating environments. The most direct form of measurement of homeostasis is the monitoring of a physiological response under changing external or internal conditions. In this work I measured the response of glycogen storage in the liver during intake of excess dietary carbohydrate. Glycogen breakdown in the liver was measured during starvation. HSI was used as an indicator of glycogen metabolism. Fish with PGM1 liver expression have a faster increase in HSI during HC feeding and a faster loss in HSI during starvation than fish lacking PGM1 expression. The liver is important in the control of blood glucose levels (Morata et al. 1982a, Morata et al. 1982b). Type B fish are more homeostatic than type A fish because of their faster response to the change in the internal availability of glucose. The faster response of carbohydrate metabolism in type B fish gives them an advantage over type A fish in the ability to adjust to varying environmental conditions.

Additional support for the greater homeostatic ability of type B fish is found when the CV is examined for several traits within each fish. Data were analysed for HSI (the relationship between LW and BW), and K (the relationship between BW and FL). The CV for HSI is lower for type B fish than type A fish in 10 of 11 comparisons (sign test,

$X^2 = 7.36$, $P < .01$; see Table 13). The CV for K was examined before and during starvation. Type B fish had a lower CV than type A fish in a significant proportion of samples (CV B<A 10/12, P = .02 using sign test; Table 14). The LM-BW relationship is more uniform in type B fish than type A fish. In addition, before and during starvation the relationship between BW and FL in type B fish shows less variance than in type A fish. This evidence suggests fish with PGM1 liver expression maintain a more constant physiological state or are more homeostatic than fish lacking PGM1 liver expression.

Table 13. Coefficient of variation for hepatosomatic index during normal and high-carbohydrate feeding. Results are expressed as percent of the mean.

		Coefficient of variation for HSI	
Family	Diet	A	B
I17	Normal 9°C	22.2	9.4
I17	Normal 13°C	9.0	14.1
J26	Normal	22.7	27.9
J26	Normal	7.5	6.7
J26	Normal	2.0	1.7
J26	HC	28.8	21.8
J26	HC	16.6	15.5
J26	HC	3.1	2.6
J27	Normal	25.1	16.5
J27	HC	26.5	19.5
J27	HC	27.5	21.1

Table 14. Coefficient of variation for condition factor during the starvation experiment. Data are from Family I17. Results are given as percent of the mean.

	Coefficient of variation for K			
	9°C		13°C	
Starvation (weeks)	A	B	A	B
0*	8.3	8.1	4.4	4.3
1	6.1	6.9	4.8	3.0
2	5.9	5.8	4.9	3.2
3	5.5	3.0	2.4	2.9
4	5.7	3.1	4.6	3.3
5	5.0	2.5	3.8	2.2

*Before starvation; normal diet.

DISCUSSION

Glycogen Metabolism

PGM1 expression in the liver has a significant effect on liver metabolism. Results of this research demonstrate that fish with PGM1 expression in the liver have a significantly greater mean HSI under normal and HC feeding conditions. Fish with PGM1 liver expression exhibit a faster gain in HSI during HC feeding and a faster loss in HSI upon starvation than fish without PGM1 expression in the liver. Changes in HSI correspond to changes in liver glycogen levels in the livers of trout and other fishes (Ottolenghi et al. 1981; Hickling and March, 1982; Hilton, 1982).

Liver glycogen levels are correlated to the changes in HSI discussed above. Fish with PGM1 liver expression tend to have greater levels of liver glycogen during normal and HC feeding than fish lacking PGM1 liver expression. The rate of glycogen storage during HC feeding tended to be greater in fish with PGM1 than fish lacking PGM1 liver expression. Fish with PGM1 liver expression showed a greater decline in liver glycogen levels during the period immediately following the start of starvation. The failure to find any statistically significant differences in glycogen levels between type A and B fish is probably explained by unavoidable handling stress during sampling. Handling stress has been shown to cause rapid changes in glycogen levels due to glycogen breakdown in rainbow trout (Nakano and Tomlinson, 1967). The affects of

handling stress may be reduced in future experiments by using training procedures (Rush and Umminger, 1978).

The effect of Pgm1 expression on liver metabolism is probably a result of Greater flux thru the pathway between G6P and glycogen. Kacser and Burns (1981), in work modeling the effect of embedding an enzyme activity variant in a multienzyme pathway, showed that steady state intermediate concentrations of any one pool will be affected by changes in every enzyme in the system. They pointed out, however, that even a 50% reduction in enzyme activity in heterozygotes for enzymatically inactive alleles is not expected to be detectable. Enzyme variants that are sufficiently different and are metabolically important should show detectable alterations in the glycolytic intermediate concentrations or energy charge (adenylate concentrations). It is possible, therefore, to regard the substrates (steady-state intermediate pools) as measurable characters. We might expect that if a difference in flux for glycogen metabolism exists between type A and B fish we should detect it in the concentrations of metabolites in the pathway.

Glycogen levels, therefore, should be indicative of any affect of PGM1 expression on flux in the pathways of glycogen storage or breakdown. As reviewed above, fish with PGM1 liver expression have greater glycogen levels during normal and HC feeding than fish lacking PGM1 liver expression. This trend for glycogen levels is supported by the results for HSI. The results for glycogen levels and HSI provide strong evidence that fish with PGM1 liver expression have greater flux through the pathways of glycogen metabolism.

Additional evidence for the affect of PGM1 expression on flux thru the pathway of glycogen metabolism comes from data on G6P levels. G6P is the substrate for PGM when the flux thru the pathway is toward glycogen storage. We would predict that type B fish would have lower G6P levels in the liver than type A fish if PGM1 expression affects flux in the pathway. Significantly lower concentrations of G6P were found in the livers of fish with PGM1 expression than fish lacking Pgm1 expression during normal and HC feeding. During starvation the flux thru the pathway is reversed. Since starved fish are breaking down glycogen reserves, the substrate for PGM is now G1P. Therefore, we would predict that during starvation G6P levels should be greater in fish with liver PGM1 expression than fish lacking PGM1 liver expression. Fish with PGM1 liver expression have significantly greater G6P levels during starvation than fish lacking PGM1 liver expression.

The above discussion supports the hypothesis of Allendorf et al. (1983a), that the presence of Pgm1 in the liver of trout embryos affects the flux thru the glycolytic pathway, resulting in an accelerated rate of development. Glycogen is the primary energy source for trout embryos and is present exclusively in the liver (see Introduction for appropriate references). Many organisms store carbohydrate in response to environmental conditions or during certain periods of their development. The accumulation and redistribution of carbohydrate materials is an important part of morphogenesis for many organisms (Pannbacher and Wright, 1967). Regulatory genes, like the Pgm1-t locus, may influence developmental rates by controlling the accumulation and breakdown of carbohydrate materials.

The effects of the Pgm1-t locus are probably not limited to those occurring during early development. PGM1 expression in the liver is likely to be important during periods of stress when glycogen reserves must be mobilized (Morata, 1982). Starvation and high temperature are two conditions where PGM1 expression is expected to be important (see Introduction). Exercise is also known to cause depletion of glycogen in liver and muscle tissue (Miller et al. 1959; Black et al. 1962). Fish with PGM1 expression in the liver may exhibit greater metabolic scope for activity than fish lacking PGM1 liver expression. Metabolic scope for activity is the difference between standard and active metabolism (Fry, 1947). Since fish with PGM1 liver expression tend to store more glycogen and can mobilize it faster than fish without PGM1 liver expression they could have a greater amount of metabolism available for work (growth, activity, reproduction). Maturation and the onset of spawning in fishes is a part of the life cycle where energy reserves must be mobilized. The high levels of protein synthesis necessary during sexual maturation, particularly in the female, is known to cause depletion of liver glycogen stores in fishes (Love, 1970; Petersen and Emmersen, 1977; Ottolenghi et al. 1981). Fish with PGM1 liver expression might cope with the physiological stress of reproduction better than fish lacking PGM1 liver expression. In addition, the eggs of fish with PGM1 expression might be affected by the increased available energy for synthetic processes during oogenesis. The potential effects of PGM1 expression during stressful conditions should provide a fruitful area for future research.

Homeostasis

Cannon (1932) defined homeostasis as the totality of steady states maintained in an organism through coordination of its complex physiological processes. Homeostasis refers to the property of the organism to adjust itself to variable conditions, or to the self-regulatory mechanisms of the organism which permit it to stabilize itself in fluctuating inner and outer environments (Lerner, 1954). This idea of physiological self-regulation is also applicable to ontogenetic processes. Developmental homeostasis or canalization of development is the buffering of the broad outlines of development against environmental disturbance, i.e. the presence of a relatively low contribution of the tangible environment to phenotypic variance (Van Valen, 1962).

Since homeostasis is a fundamental phenotypic characteristic of all living organisms much work has been done to better understand its genetic basis. Lerner (1954) proposed that heterozygosity at structural loci provide an increased ability to compensate for environmental and genetic variability during development. He suggested that more heterozygous individuals should have increased developmental homeostasis and thus be nearer to the phenotypic norm of the population. Recent work has suggested that variation in continuous traits is not necessarily determined by many structural genes each with a small effect. The continuous variation observed in some metric traits may be due to one or very few regulatory genes (Ayala and McDonald, 1980; Lehrhahn and Allard, 1965).

Measurements of developmental homeostasis include the variance at individual metric traits or the variance of several phenotypically

correlated traits within individuals. Thoday (1953) suggested that organisms may adapt to varying environments by varying their phenotype adaptively, but they must, in the process, maintain a certain harmonious balance between their component parts. Lewontin (1956) reported that the phenotypic correlation between parts of Drosophila was greater in heterozygotes than in homozygotes. HSI and K were used as measures of homeostasis in my work because they give a useful index of the relationship between component parts.

Results of this research suggest that single regulatory genes, such as Pgm1-t, may have a significant effect on developmental homeostasis. Fish with PGM1 liver expression are more developmentally buffered and homeostatic than fish without PGM1 expression. Fish with PGM1 liver expression have a lower CV for HSI and K than fish without PGM1 expression. We can better understand the origins of these organismal homeostatic effects by examining the physiology of the liver in fish with and without PGM1 expression. Liver tissue in fish with PGM1 expression responds more rapidly to changes in the internal environment (carbohydrate metabolism) than fish lacking PGM1 expression. This is possible because the increased level of PGM activity in individuals with PGM1 liver expression allows a greater flux through the pathways of glycogen storage and breakdown.

Aquaculture

Type B fish may have considerable potential in aquaculture. Type B fish may be able to be grown on HC diets or at increased temperatures with substantially reduced costs. Carbohydrates are the cheapest source of food energy. Research on rainbow trout shows that excess dietary carbohydrate has a sparing effect on protein utilization (Bergot, 1979b; Pieper and Pfeffer, 1980; Hilton, 1982). The literature shows conflicting conclusions with regard to the feasibility of HC feeding. Diets high in digestible carbohydrate have been found to be harmful to trout producing low growth and mortality (Phillips et al. 1948; NRC, 1973; Bergot, 1979a; Hilton, 1982). Some researchers, however, have reported that rainbow trout perform well on HC diets (Bergot, 1979b; Luquet, 1971; Tiews et al. 1976; Pieper and Pfeffer, 1979 and 1980; Rychly and Spannhof, 1979). In comparison to mammals, fish are relatively inefficient in using dietary glucose (Lin et al. 1978; Cowey and Sargent, 1979). Fish with PGM1 expression in the liver may be able to grow and survive on HC diets because of more efficient processing of dietary carbohydrate. Reinitz et al. (1978) pointed out that as the availability of diets high in animal protein diminishes in the future it will become increasingly important to develope strains of rainbow trout capable of using alternative diets.

Type B fish may be able to be reared at higher temperatures. Type B fish are expected to be more resistant to stress at higher temperatures than type A fish (the normal rainbow trout genotype). Type B fish may grow faster at higher temperatures thereby reducing production costs. Carbohydrate utilization has been shown to be greater

at higher temperatures (Hilton, 1982). HC feeding may be used in conjunction with higher temperatures to take advantage of the enhanced carbohydrate metabolism of type B fish. There is preliminary evidence that fish with PGM1 liver expression are more resistant to higher water temperature than fish lacking PGM1 liver expression. Allendorf (unpublished data) has detected the Pgm1-t(b) allele in natural populations of rainbow trout in the Snake River drainage of southwestern Idaho. These populations are found in streams that originate in the Owyhee Mountains and flow through the desert shrub, Owyhee lava plateau before entering the Snake River. Rainbow trout in these streams occur in areas where the water temperature sometimes exceed 28°C. Populations were sampled in water temperatues ranging from 11°C to 18°C. Fish with PGM1 liver activity had a significantly greater mean condition factor than fish lacking PGM1 liver expression in the 18°C water. In the cooler 11°C water no significant difference in condition factor was found between fish with and without PGM1 liver expression. The lower condition factor of fish lacking PGM1 liver expression suggest that they are less resistant to the stress caused at higher temperature than fish with PGM1 liver expression. This evidence from natural populations support the feasibility of rearing fish with the Pgm1-t(b) allele at higher temperatures. Also, type B fish may be useful in stocking streams where water temperatures are marginal for trout survival. Future research should test the potential advantages in aquaculture for rainbow trout with the Pgm1-t(b) allele.

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