Hepatic molecular conversion and detoxification of ferritin iron in adult lampreys (*Geotria australis*), following natural and induced iron loading

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Weekly intramuscular injections of 3 mg of iron as horse spleen ferritin into adult *Geotria australis* over 10 weeks, resulted in a progressive increase in that form of iron in the serum. However, as with control animals, the ferritin in the liver of injected lampreys consisted of one subunit type, whose M_r (20300) differed from those of the two subunit types of horse spleen ferritin. Thus, lampreys had converted horse spleen ferritin iron into endogenous ferritin iron, presumably in their liver. Marked rises in hepatic non-haem iron during the first 2 weeks and between weeks 8 and 10 of iron injections were accompanied by pronounced increases in superoxide dismutase (SOD) activity. This rise, which parallels the rise in SOD activity that occurs as

iron increases during the very protracted upstream migration of *G. australis*, is consistent with the view that SOD protects against iron-mediated damage by removing the superoxide radical, which facilitates the formation of the highly toxic hydroxyl radical. A levelling off of the iron concentration between weeks 2 and 8 was accompanied by a decline in SOD activity, even though non-haem iron levels were well above those of control animals. Enhanced SOD activity may therefore only be required when there is an elevated flux of iron in the liver through low-molecular-mass intermediates. A small amount of ferritin iron was converted into the more inert haemosiderin iron.

INTRODUCTION

The lampreys (Petromyzontiformes), together with the hagfishes (Myxiniiformes), are the sole surviving representatives of the agnathan (jawless) stage in vertebrate evolution (Hardisty, 1982). The larvae (ammocoetes) of all species of lampreys are confined to fresh water, where they live in burrows and feed on algae, detritus and micro-organisms (Moore and Mallatt, 1980). The ammocoete eventually undergoes a radical metamorphosis into a young adult which, in the case of anadromous parasitic species, then migrates downstream to the sea where it feeds predominantly on teleost fish (Beamish, 1980). When fully grown, the adult lamprey ceases feeding and embarks on a migration to the upstream regions of rivers where it spawns and dies (Hardisty and Potter, 1971).

The blood and nephric fold of ammocoetes of the Southern Hemisphere lamprey *Geotria australis* contain exceptionally high levels of iron (Macey et al., 1982a,b; Smalley et al., 1986). During the metamorphosis of this species, the amount of iron in the liver rises, reflecting the accumulation of iron released from the nephric fold and from the breakdown of larval haemoglobins (Smalley et al., 1986).

Although iron is an essential nutrient of vertebrates (Harrison et al., 1991), it is toxic when in excess (Jacobs, 1980). It has been proposed that this toxicity is in part related to the production of the extremely reactive hydroxyl radical that is formed by the interaction between 'catalytic iron' (similar to that found in low-molecular-mass complexes) and hydrogen peroxide (Halliwell and Gutteridge, 1992). This latter process may be facilitated by the superoxide radical, which has been shown by experiments *in vitro* both to convert Fe(III) into the far more soluble Fe(II) and to mobilize this metal from certain ferritins (Biemond et al., 1986; Bolann and Ulvik, 1987; Harris et al., 1994). The toxic effects of superoxide are ameliorated by its conversion into

hydrogen peroxide through the action of the enzyme superoxide dismutase (SOD: EC 1.15.1.1) (McCord and Fridovich, 1969). This presumably accounts for the increase in the activity of SOD that occurs in the liver of metamorphosing *G. australis* as the concentration of iron in that organ rises (Harris et al., 1990).

Iron loading is accompanied by a change in the form in which iron is predominantly stored (Selden et al., 1980). Thus, during iron loading in humans, the major iron-storage protein changes from ferritin to haemosiderin (Selden et al., 1980), which is less susceptible to chelator- and superoxide-induced iron release (O'Connell et al., 1986, 1989). In lampreys, the amount of iron sequestered as haemosiderin, both in the whole body during larval life and in the liver during metamorphosis, rises markedly as the total amount of stored iron increases (Macey et al., 1985; Smalley et al., 1986). The ratio of light-(L) to heavy (H) -subunits of ferritin in mammals also increases in response to iron loading (Bomford et al. 1981). Since ferritins that are rich in L-subunits have a lower turnover than those which are rich in H-subunits (Bomford et al., 1981), the shift towards a greater proportion of the light form may help reduce the potential for damage caused by high iron levels (Tran et al., 1990).

The initial results of the present study showed that, on the basis of measurements made at six monthly intervals during the spawning run of *G. australis*, the rise that occurs in hepatic iron concentration during the very protracted upstream migration of this species is accompanied by an increase in hepatic SOD activity. The study therefore then focused on elucidating the pattern and rapidity of the response by SOD in the liver of *G. australis* to increased hepatic iron produced through weekly intramuscular injections of horse spleen ferritin. The form in which ferritin iron was sequestered in the liver was also investigated to ascertain the extent to which adult lampreys are able to convert ferritin iron into haemosiderin iron and thereby help counteract the toxic effects of this element.

MATERIALS AND METHODS

Maintenance and treatment of animals

Adult *G. australis* were collected during the early part of their spawning run in the Margaret River in south-western Australia (34°S, 115°E). They were maintained in aquaria exposed to temperatures and photoperiod that were regularly adjusted so that they paralleled those occurring in the field. The animals were acclimated to the laboratory regime for 6–8 weeks before the commencement of the experiment.

Since the injection of iron dextran proved fatal to lampreys, horse spleen ferritin was chosen as the vehicle for iron loading in these animals. Horse spleen ferritin was purified using the method of Harrison et al. (1980). The purified ferritin was precipitated by ultracentrifugation at 217 000 *g* for 2 h using a Beckman L8-70 ultracentrifuge equipped with a 70.1 Ti rotor. The pellet was gently washed in 0.15 M NaCl and then redissolved overnight in the same medium. The resulting solution was centrifuged at 8130 *g* for 5 min in an Eppendorf model 5414 centrifuge to remove undissolved ferritin. The pH was adjusted to 7.0 using 0.5 M NaOH and the solution was sterilized using a 0.22 µm filter. The iron content of this ferritin was determined after digestion in HCl/trichloro-acetic acid (TCA) using the assay of Yee and Goodwin (1974) as described below.

On day one of the experiment, 30 animals were injected with 3 mg of iron as horse spleen ferritin, while a further five animals were injected with the equivalent volume of 0.15 M saline and then killed. After 1 week, five of the injected animals were killed and the remaining 25 animals were again injected with 3 mg of iron. Weekly injections of 3 mg of iron were continued, with a further five animals being sacrificed at the end of each of weeks 2, 4, 6, 8 and 10. Thus, after weeks 1, 2, 4, 6, 8 and 10, the sacrificed animals had received cumulative injections of 3, 6, 12, 18, 24 and 30 mg of iron, respectively.

Preparation of samples

Lampreys were anaesthetized with 0.01% benzocaine. Blood was collected by cardiac puncture and stored on ice until centrifugation at 1000 *g* for 10 min to provide serum, which was then frozen at –20 °C. The animal was killed by decapitation and the liver was removed and macerated with a razor blade. A random sample of the macerate was homogenized in 9 vols of ice-cold phosphate buffered saline, containing 0.15 M NaCl/0.1 mM EDTA/50 mM K₂HPO₄, pH 7.8. A portion of this homogenate was further diluted 1:9 with deionized water and frozen at –20 °C for analysis of total and ferritin iron. The remainder was centrifuged for 35 min at 217 000 *g* in a Beckman L8-70 ultracentrifuge using a 70.1 Ti rotor. The supernatant was removed and dialysed overnight as described previously (Harris et al., 1990). The protein content of each supernatant was measured before and after dialysis, using the method of Bradford (1976). The ratio of protein concentrations, before and after dialysis, was multiplied by the SOD activity that was subsequently determined, thus compensating for any dilution of the cytosol that might have occurred during dialysis.

SOD assay

SOD assays were performed using a modification of the cytochrome *c* method of McCord and Fridovich (1969). Reaction mixtures contained 50 mM K₂HPO₄, 0.1 mM EDTA, 40 µM cytochrome *c* and 0.2 mM xanthine (pH 7.8). Reactions were initiated by the addition of sufficient xanthine oxidase to produce, in the absence of SOD, a change in absorbance of approximately 0.025/min at 550 nm. Incremental volumes of cytosol were

assayed and the activity of SOD was calculated from the slope of the relationship between SOD activity and the volume of cytosol added. Results are expressed as units of SOD activity, where one unit of SOD is the amount of enzyme required to halve the rate of cytochrome *c* reduction under the conditions specified by McCord and Fridovich (1969).

Iron assays

Total and ferritin iron were separated using the method of Kaldor (1958). Samples frozen at the time of sacrifice were thawed and duplicate 400 µl aliquots were taken for total iron assay. The remainder of the thawed samples was centrifuged in an Eppendorf model 5414 centrifuge at 8130 *g* for 5 min and duplicate 400 µl aliquots of the supernatant taken for ferritin iron assay. Thereafter, both the total iron and the ferritin iron samples were treated identically. Samples were heated at 90 °C with 280 µl of 10% HCl for 30 min, followed by a further 20 min of heating after the addition of 120 µl of 33% TCA. After centrifugation at 8130 *g* for 5 min in an Eppendorf centrifuge, duplicate 400 µl portions of the supernatants were taken for iron assay using a modification of the method of Yee and Goodwin (1974). The concentration of haemosiderin iron was determined by subtracting the concentration of ferritin iron from that of total iron.

PAGE analyses

Serum and liver samples were prepared for PAGE analyses in the following manner. The serum was thawed, mixed with glycerol (20%), running buffer (40%) and Bromophenol Blue (0.4 mg/ml) and centrifuged for 3 min at 8130 *g* in an Eppendorf centrifuge. Samples of frozen liver were thawed and homogenized in 14 vols of Tris-glycine running buffer (25 mM Tris/192 mM glycine, pH 8.3) to which 0.12 mM phenylmethanesulphonyl fluoride (PMSF) had been freshly added as protease inhibitor. This homogenate was centrifuged at 8130 *g* for 3 min in an Eppendorf centrifuge. The resulting supernatant was heated for 10 min at 70 °C, chilled in an ice-bath for 10 min, and finally centrifuged for 5 min at 8130 *g*. A portion of the resulting supernatant was mixed with 20% glycerol, 40% running buffer and 200 µg of Bromophenol Blue and used for non-dissociating (native) PAGE, while the remainder was loaded on to a linear gradient of sucrose (0–2.5 M) and centrifuged for 6 h at 217 000 *g*. The upper layers of the supernatant were removed and the broad brown, ferritin-containing band was collected and passed down a Sephacryl S300 molecular-exclusion column (Pharmacia), using Tris-glycine buffer (25 mM Tris/192 mM glycine, pH 8.3) as eluant. The brown, ferritin-containing fractions were collected and assayed for protein content (Bradford, 1976). The purified ferritin from iron-loaded lamprey livers, together with those extracted from control lamprey livers and from horse spleen, were dissociated into subunits by adding 25 µg of ferritin protein to 10% glycerol, 5% 2-mercaptoethanol, 2% SDS and 120 µg of Bromophenol Blue and heating to 95 °C for 10 min, before being analysed using SDS/PAGE.

Non-dissociating PAGE analysis of plasma and liver homogenate samples were performed using a resolving gel of 5% and a stacking gel of 2.5%, with a Tris-glycine running buffer, pH 8.3 (Hames and Rickwood, 1981). Wells contained 20 µl of liver sample, 20 µl of serum sample or standards of lamprey or horse spleen ferritin. Gels were loaded into a MiniProtean II electrophoresis unit (BioRad, CA, U.S.A.) submerged in an ice-bath and then run at 50 V for 30 min followed by 75 V for 2.5 h. The gels were then removed and stained for 30 min with 1% potassium ferrocyanide in 1% HCl made immediately before use

(Perls' reaction), and flushed with running tap-water overnight to remove unreacted Perls' reagent.

SDS/PAGE was performed using a 12.5% acrylamide resolving gel, containing 1% SDS in 350 mM Tris, pH 8.3, and a stacking gel consisting of 5% acrylamide containing 1% SDS in 125 mM Tris, pH 6.8 (Hames and Rickwood, 1981). Wells contained 5 μ g of ferritin subunits, or standards consisting of BSA (3.5 μ g), egg albumin (5 μ g), carbonic anhydrase (2.5 μ g), trypsinogen (5 μ g), trypsin inhibitor (5 μ g) and α -lactoglobulin (2.5 μ g) which had been dissociated as before. Gels were run for 1.5 h at 150 V, after which they were stained with Coomassie R250 dye in 40% ethanol/10% acetic acid.

RESULTS

Natural iron loading

The mean non-haem iron concentration in the liver of *G. australis* rose from 0.43 mg/g in August 1992, just after the commencement of the spawning run, to 0.74 mg/g in the following February and 2.0 mg/g in August 1993. The contributions of haemosiderin to the total iron were 5, 7 and 14%, respectively. SOD activity showed a similar progressive increase to that of total non-haem

iron, with corresponding values of 1388, 2584 and 3188 units/g of liver at the three times during the spawning run.

Experimental iron loading

The mean total iron concentration in the serum rose from 1.9 mg/l in control animals to 9.4 mg/l after 10 successive weeks of injections of horse spleen ferritin (Figure 1). PAGE revealed that the major non-haem iron component in serum co-migrated with the main component of horse spleen ferritin (Figure 2). An additional minor band, which migrated in a position comparable with those assumed to be horse spleen ferritin dimers (see Figure 2), was evident from week 4 onwards.

The mean concentration of non-haem iron in the liver of the control group that was killed at the beginning of the experiment was 0.74 mg/g (Figure 3). In contrast, the mean concentration in the liver of animals that had been sacrificed 1 week after injection with 3 mg of ferritin iron was 2.3 mg/g, i.e. more than three times the level of the control. The mean concentration rose slightly between the end of weeks 1 and 2, before declining to 2.1 mg/g at the end of week 4. Concentrations then rose slightly to 2.6 mg/g at week 8 and then sharply to 5.5 mg/g at week 10 (Figure 3).

The trends exhibited by mean SOD activity initially paralleled those of total non-haem iron (Figure 3). Thus, the mean SOD activity in the liver of animals injected weekly with 3 mg of iron had increased from 2584 units/g in the control to 3908 and 5440 units/g at weeks 1 and 2 respectively. Although the trends shown by SOD activity then followed those of iron concentration

Figure 1 Mean concentrations $\pm$ S.E.M. for total non-haem iron in the serum of adult *G. australis* after the animals had received weekly injections of 3 mg of horse spleen ferritin iron

Control animals (week 0) received no injections of iron. Five animals were sacrificed at each of the weeks indicated.

Figure 2 Native PAGE analysis of purified horse spleen ferritin (lane a) and control lamprey liver ferritin (lane b) and of the serum of *G. australis* after the animals had received weekly injections of 3 mg of horse spleen ferritin iron and were sacrificed at weeks 0, 2, 4, 6, 8 and 10 respectively (lanes c–h)

Control animals (lane b) received no injections. After electrophoresis, the gel was stained to reveal the iron using Perls' reagent.

Figure 3 Mean values $\pm$ S.E.M. for the concentrations of total iron and activities of SOD in the liver of adult *G. australis* after the animals had received weekly injections of 3 mg of horse spleen ferritin iron

Control animals (week 0) received no injections of iron. Five animals were sacrificed at each of the weeks indicated.

Figure 4 Native PAGE analysis of purified horse spleen ferritin (lane a) and control lamprey liver ferritin (lane b) and of liver extracts of *G. australis* after the animals had received weekly injections of 3 mg of horse spleen ferritin iron and were sacrificed at weeks 0, 1, 2, 4, 6, 8 and 10 respectively (lanes c–i)

Control animals (lane b) received no injections.

by falling slightly at week 4, SOD activity subsequently continued to decline to 2936 units/g at week 6. However, SOD activity rose sharply to 4768 units/g after week 10, mirroring the sharp rise in hepatic iron levels over this period (Figure 3).

Native PAGE of liver homogenates revealed that, irrespective of the liver iron content, the main iron-containing protein in the lamprey liver had a different relative mobility to those of either the monomeric or oligomeric components of horse spleen ferritin (Figure 4). The major iron-containing band co-migrated with the lamprey liver ferritin which had previously been extracted from the livers of uninjected adult lampreys and purified to homogeneity. Isoelectric focusing yielded values of ≈ 5.5 for lamprey ferritins from each week, compared with ≈ 4.8 for the main horse spleen ferritin.

SDS discontinuous PAGE showed that the ferritin from iron-

loaded lampreys consisted of a single subunit of M_r 20300 (Figure 5). This value is identical with that obtained using ferritin from animals that had not been injected with horse spleen ferritin (Figure 5). In contrast, horse spleen ferritin consisted of two types of subunit, consistent with the results of Arosio et al. (1978).

The mean concentration of the haemosiderin component of the iron after 4 weeks of injections was still similar to that of the control i.e. 0.05 mg/g (Figure 6a). It rose slightly in the next 4 weeks and then sharply to 0.39 mg/g between weeks 8 and 10 (Figure 6a).

The percentage contribution that haemosiderin iron made to total hepatic iron content declined significantly ($P < 0.05$) from 7.4% in the control animals to a minimum of 2.6% at week 4, then rose significantly ($P < 0.05$) to 6.3% at week 10 (Figure 6b).

DISCUSSION

Uptake of iron and conversion of horse spleen ferritin

The present study has shown that weekly injections of horse spleen ferritin into the musculature of adult *G. australis* result in a progressive rise in that iron component in the serum of these animals. Our results also demonstrate, however, that the ferritin found in the liver during the 10 week experiment was always lamprey ferritin. Since the amount of ferritin in the liver at the end of the injection period was over seven times that at the beginning of the experiment, the lamprey has clearly converted a substantial amount of injected horse spleen ferritin iron into the lamprey analogue.

Although iron levels rose in the liver between the commencement of the experiment and the end of the second week after injection, and also between the end of weeks 8 and 10, they remained relatively stable in the intervening period. This was despite the fact that iron levels in the serum rose progressively over the 10 week period. The above results suggest that the amount of iron presented to the liver between weeks 2 and 6 may exceed, at that time, the capacity of the liver to process any further iron. The rise in hepatic iron between weeks 8 and 10 occurred when the amount of haemosiderin had increased sharply. From the results of a previous study (Macey and Youson, 1990), this implies that ferritin iron may be becoming progressively incorporated into lysosomes and then converted into haemosiderin. This would thus effectively be lowering the cytosolic iron level and thereby increasing the potential for more iron to be taken up.

Although ferritin is present in high concentrations in the sera of both larval lampreys (Macey et al., 1982b) and in humans with iron-overload disease (Pootrakul et al., 1988), and can thus presumably function as a transport molecule, our study has shown that endogenous ferritin does not play this role to any extent in the serum of adult lampreys, even when they have been subjected to iron overload. The absence of lamprey ferritin in the sera of the control and all injected lampreys, combined with the presence of iron as lamprey but not horse spleen ferritin in the liver, implies that the pronounced increase in lamprey ferritin that occurs in the liver of lampreys following intramuscular injection of horse spleen ferritin is the result of enhanced hepatic synthesis of this protein. The iron that led to the increased hepatic levels of this metal would have been derived either directly or indirectly from horse spleen ferritin. Any direct derivation would presumably involve an uptake of the horse spleen ferritin by the liver and the subsequent transfer of its iron to the endogenous protein, a transfer which must have been complete within 1 week of each injection. Alternatively, any indirect derivation would involve release of iron from the horse

Figure 5 SDS discontinuous dissociating PAGE analysis of purified horse spleen ferritin (lane a) and control lamprey liver ferritin (lane b) and of liver extracts of *G. australis* after the animals had received weekly injections of 3 mg of horse spleen ferritin iron and were sacrificed at weeks 2, 4, 6, 8 and 10 respectively (lanes c–g)

Control animals (lane b) received no injections.

Figure 6 Mean values $\pm$ S.E.M. for (a) the concentration of haemosiderin and (b) the percentage contribution of haemosiderin to total non-haem iron in the liver of *G. australis* after the animals had received weekly injections of 3 mg of horse spleen ferritin iron

Control animals (week 0) received no injections of iron. Five animals were sacrificed at each of the weeks indicated.

spleen ferritin in some other tissue site and its transportation to the liver bound to transferrin, the typical iron transport molecule in the serum of adult lampreys (Macey et al., 1982b).

Ferritins are a heterogeneous family of proteins whose subunit composition varies between tissues (Arosio et al., 1978) and with degree of iron loading (Kohgo et al., 1980). Throughout our experiment, the ferritin present in the lamprey liver consisted of a single type of subunit, contrasting with the situation in the liver of humans (Tran et al., 1990) and rats (Bomford et al., 1981), where the ferritins consist of varying proportions of H- and L-subunits. In the case of human and rat liver, increasing concentrations of iron are accompanied by a shift from the H- to L-subunit. Since ferritins that are richest in L-subunits are degraded much more slowly than those in which the H-subunit predominates (Bomford et al., 1981), and since the whole ferritin shell appears to be metabolized as a unit (Bomford et al., 1981), it is almost certain that the turnover of iron from L-subunit-rich ferritins is also slower. However, since the ferritin of *G. australis* releases its iron more slowly than those of other vertebrates (Harris et al., 1994) there would be no obvious advantage in lampreys having the ability to undertake a similar type of molecular shift.

Relationships between SOD activity and increasing iron levels

The fact that iron was released from horse spleen ferritin and incorporated into lamprey ferritin indicates that the iron must at least transiently have entered the highly reactive, low-molecular-mass pool in the liver cells. At such times, the iron would be likely to lead to the generation of toxic hydroxyl radicals (Halliwell et al., 1992). The progressive increase in hepatic SOD activity, which accompanied the rise in total hepatic non-haem iron that occurred during the protracted spawning run of *G. australis*, was paralleled by the increases in SOD activity in the liver when iron levels rose in response to intramuscular injections of iron. The activity of the superoxide radical detoxifying enzyme thus rose most sharply between weeks 0 and 2 and between weeks 8 and 10 of induced iron-loading. This result also parallels the situation in the liver of *G. australis* during metamorphosis, when the level of SOD activity increases in concert with rises in iron flux (Harris et al., 1990). These responses by SOD would help minimize the superoxide-mediated reduction of iron to the reactive divalent form, which is involved in the formation of toxic hydroxyl radicals from hydrogen peroxide (Halliwell et al., 1992).

Although hepatic SOD activity increased when iron concentrations in the liver rose, the essentially static hepatic iron level recorded between the end of weeks 2 and 6 after injection of iron was accompanied by a decline in SOD activity. This decline suggests that SOD activity tracks the hepatic flux of iron through low-molecular-mass intermediates rather than following the absolute levels of iron in the liver. In other words, the reduction in SOD activity between weeks 2 and 6 is seen as being related to a reduction in the transient and most reactive forms of iron in the liver during this period.

While there must be a flux of iron through the low-molecular-mass pool at times when hepatic lamprey ferritin levels are increasing, the characteristics of lamprey ferritin would ensure that any such flux is minimal when iron levels are constant. Thus, in comparison with the ferritins of other vertebrates, that of the lamprey is not only very efficient at binding iron but is also not susceptible to superoxide-mediated iron release (Harris et al., 1994). This would further explain why, after 6 weeks of injections, when the iron levels are still far in excess of control

animals but remaining relatively constant, SOD activity declined back to basal levels.

Incorporation of iron into haemosiderin

The amount of iron sequestered as haemosiderin by animals, including larval lampreys, often increases markedly as iron concentrations rise (Selden et al., 1980; Macey et al., 1985; St. Pierre et al., 1987). Since, at least in the horse, haemosiderin releases its iron less readily than ferritin, the incorporation of iron into the former of these two storage molecules is likely to help protect the animal from the toxic effects of this metal (O'Connell et al., 1989). In the case of adult *G. australis* subjected to injections of exogenous iron, the percentage of iron sequestered in the liver as haemosiderin, fell precipitously during the first 2 weeks following injections and then rose progressively between weeks 4 and 10. This implies that the injected iron is initially sequestered as lamprey ferritin iron and only after 4 weeks of injection is there a conspicuous trend for hepatic ferritin iron to be converted into haemosiderin iron. This conclusion is consistent with the progressive upward trend in the mean concentration of haemosiderin in the liver between weeks 4 and 10, albeit slow between weeks 4 and 8. Although haemosiderin iron still only contributed 6.4% to the total amount of iron in the liver after 10 weeks, the fact that there is a delay in the conversion of ferritin iron into haemosiderin iron and that the amount of haemosiderin rose sharply between weeks 8 and 10, suggests that an increasing amount of iron would have been sequestered as haemosiderin iron in the ensuing weeks, had the experiment continued. Such a conclusion would be consistent with the presence of a greater proportion of haemosiderin iron at the end of the spawning run than after 10 weeks of injections (14% versus 6.4%) even though the concentrations of total non-haem iron were lower than at the end of the injection period. Since the proportion of hepatic iron in the form of haemosiderin iron is still small however at the end of the spawning run, the restricted amount of ferritin iron that is converted into haemosiderin iron in the liver of adult lampreys probably provides only limited protection against the potentially toxic effects of this metal.

In summary, a certain amount of the horse spleen ferritin iron that was injected into the musculature of adult *G. australis* entered the serum of those animals. The iron of this ferritin was transferred to lamprey ferritin, which consequently led to an increase in the concentration of iron bound to lamprey ferritin in the liver. Increases in hepatic iron were accompanied by an elevation in the activity of SOD, which presumably minimizes the interaction of iron with superoxide and thus renders it less toxic. The small proportion of ferritin iron that was converted into haemosiderin iron would provide some further limited protection.

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