Aspects of iron metabolism in a freshwater mussel

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Estimations of the total iron content of tissues of *Elliptio* confirm that this animal concentrates iron. During starvation, tissue mass decreases more than total iron and an apparent increase in iron content results. Most iron is contained in the viscera and mantle, while muscular tissue contains least.

^{59}Fe injected into the visceral mass was rapidly translocated to the gills and mantle where much of it accumulated in tissue that acted as a 'kidney of accumulation'. The ^{59}Fe did not become fully incorporated into the physiological iron pool in 10 days, nor was it rapidly excreted. There is little metabolic turnover of elemental iron.

This iron store is much greater than needed in known enzyme systems. It may indicate hyperactivity of an uptake mechanism or the lack of an adequate excretory mechanism.

Introduction

The bivalve molluscs have long been known as accumulators of various elements (Vinogradov 1953).

Dubuisson and van Heuverswyn (1931) made a chemical and histochemical study of iron and manganese in the gills of the freshwater mussel *Anodonta cygnea*, and reported the presence of distinct granules containing these elements. The review by Bunting (1949) of techniques for iron histochemistry indicates that their methods may not have been adequate. Bowen (1949), working on various North American species, did not confirm all of their observations, although it is true that the gills of freshwater mussels are rich in iron. Bowen analyzed small numbers of individuals from a variety of localities, and noted considerable individual variation in his samples. Some of this was probably caused by the use of freshly collected animals. For *Mytilus edulis* Hobden (1967) showed that undigested food, which required at least 2 days for total elimination, could produce exceptionally high readings. The same study also showed that the iron stores of *Mytilus* decreased steadily under conditions of starvation until a stable level (permanent store) was reached. The present study was intended to extend work done on the freshwater unionids and on *Mytilus*, particularly to check on the existence of a permanent store in a unionid and to try to obtain information on its metabolic turnover. Attempts at iron histochemistry by use of the methods recommended by Bunting (1949) had proved relatively fruitless with *Mytilus* so autoradiography was used as an alternative technique (Hobden 1969*b*).

Methods

The species used was *Elliptio complanata* (Solander), from the Ottawa River. Dr. A. H. Clarke of the National Museum of Natural Sciences, Ottawa, kindly made the initial identification.

Dissection of Specimens

First the shell valves were pegged open and the fluid allowed to drain from the mantle cavity. The adductor muscles were carefully cut with a stainless steel scalpel and one valve was removed. All other dissection was done with non-ferrous instruments. The whole of the soft tissue of each animal was divided into mantle, plus palps; gills; digestive gland, including stomach; visceral mass, including most of the intestine and gonad and some foot muscle; foot (ventral part only); adductor muscles; and the pericardial complex, including heart, part of the rectum, and much of the renal organ. Blood was allowed to drain into the large petri dish used for dissection and then collected. This resulted in a certain amount of dilution as well as contamination by tissue debris but gave much better recoveries than other techniques. No blood was collected for the purely chemical studies after it was established that uncontaminated blood contained only a few micrograms of iron per animal. Each tissue sample (other than blood) was blotted dry with filter paper and weighed in a tared digestion flask.

Iron was estimated colorimetrically with 1:10 phenanthroline after the method of Sandell (1950), following digestion of the tissues by mixed nitric and sulfuric acids.

Radioactive iron, ^{59}Fe, was obtained as $^{59}FeCl_3$ in hydrochloric acid from Atomic Energy of Canada Ltd. Solutions for injection were prepared in neutral citrate buffer with the addition of inactive iron and an approximately equivalent amount of dilute sodium hydroxide to neutralize the acid in the stock solution. These solutions were prepared as required and used immediately. One- to three-microcurie quantities of ^{59}Fe (specific activity about 1 μcurie/μg) were then injected directly into the

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visceral mass regions of the experimental animals using a 1½ or 2 in. × 24 gauge needle inserted via the antero-ventral region of the foot muscle, to minimize leakage as the needle was withdrawn. Gentle handling of the animals allowed the needle to be inserted between the valves in most cases before the shell was tightly closed.

The total activity in the tissue samples was counted in a single-channel well-type crystal scintillation system set to accept both of the ^{59}Fe gamma rays. For each tissue a specific activity value was calculated in terms of counts per minute per microgram of iron. The same calculation was made for the whole animal using the totals of counts per minute and iron content for each tissue. Then all tissue results were expressed in terms of multiples of this whole animal value as specific activity ratios. A ratio of one indicates that the tissue contains equal fractions of the total radioactive and total non-radioactive iron. Deviations from a value of one indicate the relative excess or deficiency of radioactive iron in the tissue.

Autoradiography

Tissues were fixed in neutral 10% formalin (Bunting 1949), wax-embedded, sectioned at 10 μ, and coated with collodion. Kodak NTB 3 nuclear emulsion was applied by the dipping method (Gude 1968). After exposure of 3–35 days, 4 min development in 50% Kodak D.19 developer at 6–10 °C gave satisfactory results without excessive background. Some of the autoradiographs were stained with hematoxylin and eosin before they were cleared and mounted.

Results

Whole Tissues

Table I compares the iron content of mussels caught 1–3 weeks earlier with that of mussels kept in an aquarium supplied only with running tap water for 6 months. In these animals the loss in mass of soft tissue is proportionally much greater than loss of iron, resulting in an increase in actual iron content.

The experiments with ^{59}Fe usually used animals that were caught 1–6 weeks before. Table II shows the specific activity ratios found in these animals for two intervals after injection. The samples were pooled in this manner when it was found individual variation obscured other trends.

The values change during the first 2 days after injection. The radioactivity may take up to 1 day to disappear from the blood. After day 2 the pattern changes only slowly if at all. The overall pattern of radioactive iron distribution can be obtained by combining these results with Table I. The gills contain a relatively high proportion of the radioactivity.

TABLE I

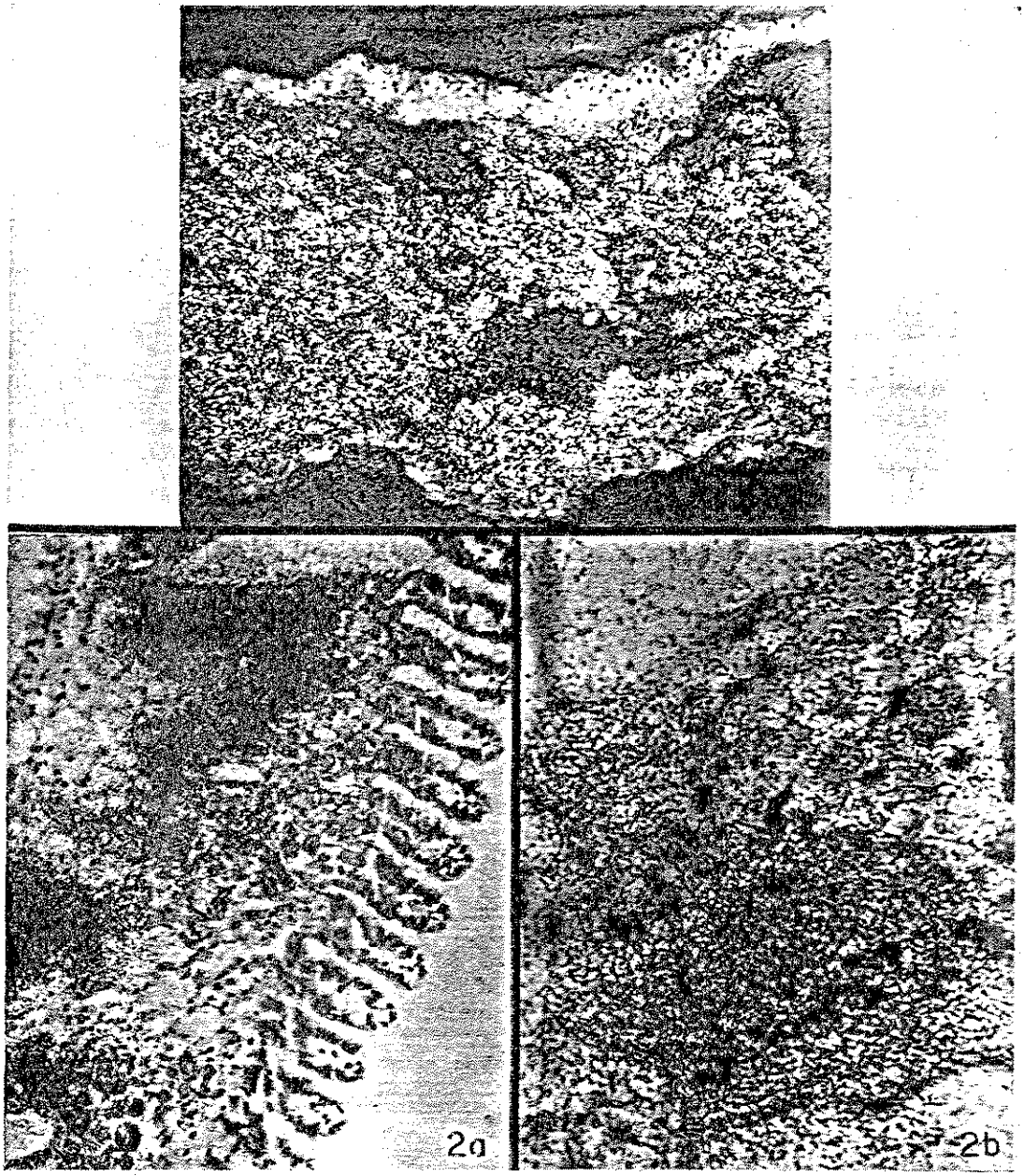
Comparison of total iron content of recently collected and starved mussels (values as μg/g) (n = 9–11 for all samples)

Tissue	"Fresh" mean ± st. dev.	"Starved" mean ± st. dev.	Significance level for difference (p <)
Adductors	21 ± 19	9 ± 3	0.05
Foot	116 ± 52	131 ± 22	—
Gills	177 ± 30	207 ± 48	0.10
Mantle	197 ± 24	233 ± 50	0.05
Visceral mass and pericardial complex	192 ± 66	412 ± 126	0.001
Digestive gland	203 ± 88	384 ± 188	0.01
Whole animal	148 ± 25	185 ± 28	0.10

TABLE II

Specific activity ratios (tissue sp. activity/whole animal sp. activity) after injection of ^{59}Fe into mussels (n = 29–34 for all samples)

Tissue	Time after injection: ½–6½ h (mean ± st. error)	Time after injection: 2–10 days (mean ± st. error)	Significance level for difference (p <)
Adductor muscles	0.71 ± 0.11	0.46 ± 0.06	0.05
Blood	11.92 ± 2.61	1.12 ± 0.15	0.001
Mantle	1.10 ± 0.07	1.34 ± 0.07	0.02
Digestive gland	0.51 ± 0.05	0.56 ± 0.07	—
Visceral mass	0.65 ± 0.07	0.50 ± 0.02	0.05
Foot	0.57 ± 0.18	0.58 ± 0.07	—
Gills	1.82 ± 0.14	1.97 ± 0.17	—
Pericardial complex	0.88 ± 0.58	1.09 ± 0.08	0.05



FIGS. 1 and 2. Autoradiographs of tissues of *Elliptio* killed 4 days after the injection of ^{59}Fe. All counterstained with hematoxylin and eosin. FIG. 1. The mantle showing radioactivity in the conjunctive tissue. (100 ×). FIG. 2*a*. Horizontal section near the base of a gill showing radioactivity in a dense tissue within the lamellae. (100 ×). FIG. 2*b*. High power of the iron-accumulating tissue in the base of the gill. (500 ×).

Other experiments indicated that the injected iron is excreted very slowly if at all.

Autoradiographs

Three series of autoradiographs were prepared from pairs of animals that had been injected with 3 μcuries of ^{59}Fe, at 1, 4, or 11 days before. Four basic tissues were examined: the mantle, gills, digestive gland, and visceral mass. There was little obvious difference in ^{59}Fe distribution in all the animals examined. The mantle contained activity within its spongy central (conjunctive) tissue but not in the epithelia nor in the muscle of the distal portion (Fig. 1). Activity was greatest in the proximal (dorsal) part of the mantle with a gradual decrease towards the distal edge. In sections which included some periostracum it, too, contained activity.

In the gills, almost all of the activity was confined to a dense pigmented tissue that penetrates the lamellae at their base (Fig. 2). It may not be renal organ tissue as such but in gross dissection it is easily associated with it (see discussion). Activity was also observed in some gill filaments, associated with the skeletal rods, and also on the walls of some blood sinuses.

Although the visceral mass was the site of the original injection, its activity was lower than the gills and mantle. There was activity in the outer layer of mantle-like tissue covering the organs, but not in the epithelium itself. There was other activity in tissues deep beneath the intestinal epithelia, and sometimes in some of the ova of females.

The digestive gland also contained relatively little radioactive iron. The glandular cells lining the tubule walls seemed to be slightly active. Deep beneath the stomach epithelium there was more activity. The most active tissue was the amorphous renal organ tissue seen in some sections.

Discussion and Conclusions

The chemical estimations confirm that there is little or no iron loss during starvation. In this example of extreme starvation the animals have depleted their general food reserves much more than their iron reserves. The iron content of *Elliptio* is some three times that of *Mytilus* (Hobden 1967).

The results with ^{59}Fe show that the injected iron is translocated by the blood stream, and fairly rapidly removed from it by certain tissues, particularly parts of the conjunctive tissue of the mantle and the interlamellar tissue of the gills. In both cases the cells seem to be a special type of pigmented cell, rather than the normal cells of the tissue. From their brownish color and ability to act as a "kidney of accumulation" these cells are comparable to the pericardial organs of other bivalves (see Franc 1960; Martin and Harrison 1966).

Pericardial gland tissue does occur in the mantle of some bivalves (White 1942) but has apparently not been reported within the gills. White also states that the pericardial gland in *Elliptio dilatata* is in the mantle anterior and dorsal to the pericardium, well removed from the gills. Both Bowen (1949) and Harrison (1966) have observed a tissue at the base of the gills in different North American species, including an *Anodonta*, but neither has named it. Harrison reported that the tissue did not accumulate iron added to the aquarium water. The tissue in the gills could be just a differentiated region of the true renal organ, while that in the mantle is more likely pericardial gland, although it is much more extensive than normal pericardial gland tissue.

The deviation of the specific activity ratios from one indicates that the iron has not completely mixed into a metabolic pool. Probably the animals normally contain iron in at least two physiological pools in different tissues: a store in what seems to be a primarily excretory tissue in gills and mantle, and another pool in the digestive gland and other viscera which may have more physiological value. To these can probably be added the excess of iron accumulated by the digestive gland during active feeding and which is readily lost on starvation (Hobden 1967).

The whole picture is of little active turnover of elemental iron as is also found in *Mytilus* (Hobden 1969*a*). There must be turnover for cell renewal but this can involve iron in combined form. The amounts in known enzymes such as cytochromes (Kawai 1961*b*) or catalase (Marks and Fox 1934; Hobden 1970) are only tiny fractions of the total iron; Kawai (1961*a*) has reported an "enterochrome" in the gut of a unionid. This might account for some more of the iron.

Bowen (1949), considering manganese, has speculated that most of the store of such elements is in a "non-metabolised" form. The organism only requires the element in trace amounts but these exceed the environmental concentration. The possession of an uptake system would be a definite selective advantage. Within limits there is no selective disadvantage if this mechanism is hypereffective. The surplus is stored. The stored form may be "insoluble" (Seah and Hobden 1969) but it could still provide the essential trace element in an emergency. A partial alternative to this explanation is that some of every naturally occurring element must enter the animal with its food. If no adequate excretory mechanism exists, as in the bivalve molluscs the element will accumulate, probably in a bound or insoluble form.

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