BIOKINETICS OF CESIUM IN *PERNA VIRIDIS*

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Abstract—The biokinetics of Cs in four compartments in the green-lipped mussel *Perna viridis,* namely, gill, viscera, adductor muscle, and foot, were studied. First-order linear differential equations were set up for these four compartments, and their solutions were used to fit the experimental data. The parameters governing the biokinetics, which depend on the elimination rate from each compartment and the transfer coefficient between compartments, were found. These are useful in understanding the physiology of *Perna viridis,* in predicting the activity of cesium in each compartment of *Perna viridis* from a contamination history, or in using *Perna viridis* as a sentinel organism for surveying and monitoring radioactive contamination. The results showed that the viscera should be represented by more than one compartment. Concentration factors for the four compartments and for *Perna viridis* were also determined, and these agreed well with reported values in the literature.

Keywords—*Perna viridis*   Biokinetics   Bioaccumulation   Concentration factor   Cesium

INTRODUCTION

Since the commissioning of the Daya Bay Power Station in 1994, additional new nuclear power plants in Guangdong, China, are under construction or being planned as China is gradually increasing its reliance on nuclear power to meet its future electricity demand. Because of the proximity of these plants, nuclear accident consequence analysis capability is needed to assess the impact of the potential severe nuclear accidents on the public health of Hong Kong. After the Chernobyl nuclear reactor accident in the former Soviet Union, people have become more aware of the health effects of artificial radionuclides discharged into the environment. Research has shown that the aquatic ecology plays an important role in the assessment of the contamination of the area and the consequences of a nuclear accident [1–3].

The biokinetics of cesium in different mussels were studied [4–6]. In the present work, the biokinetics of Cs in four compartments in the green-lipped mussel *Perna viridis,* namely, gill, viscera, adductor muscle, and foot, were studied. The green-lipped mussel *Perna viridis* (Linnaeus, 1758) (Bivalvia: Mytilacea) is widely distributed in tropical and subtropical Asia and has a northern limit at southern Japan [7,8]. Monthly growth rates of this species can exceed 10 mm during the first year in warm waters, with typical adult shell length ranging from 10 to 12 cm. These animals are widely cultivated as food in China and Southeast Asian countries, such as the Philippines, India, Singapore, and Thailand [9].

These mussels possess many characteristics that make them an ideal pollution indicator [10]. Specifically, these filter feeders tend to bioaccumulate pollutants, especially metals, in their tissues, and their body burdens of trace metals have often been used to reflect levels of contamination in surrounding waters. Nevertheless, it is important to note that *P. viridis* has the ability to regulate certain metals (e.g., zinc) in their soft tissues [10,11]. Indeed, the uptake and depuration kinetics may be

different for various metals, reflecting the bioavailability and bioreactivity of individual metal species.

The results from the present work are useful in understanding the physiology of *Perna viridis,* in predicting the activity of cesium in each compartment of *Perna viridis* from a contamination history, or in using *Perna viridis* as a sentinel organism for surveying and monitoring radioactive contamination.

EXPERIMENT

The mussels for the experiments were collected from fish farms in Kat O of Hong Kong. All the collected mussels had a size between 8 and 10 cm. They were brought back to the laboratory for acclimation and were evenly distributed in four tanks for 5 d. During the acclimation period, the tanks were filled with clean, filtered seawater with aeration and a water temperature of 27 °C, and a florescent light was set over the tanks and switched on with a 12:12-h LD (light:dark) cycle.

After acclimation, the mussels were transferred to three tanks, each containing 10 L of filtered seawater (temperature, 27 ± 1°C; salinity, 32 ± 1‰; pH, 7.8; DO, 7 ± 1 mg/L); sufficient Cs was added to each tank so that the final concentration of Cs was 5 µg/ml. The tanks were aerated and maintained on a 12:12-h LD cycle.

Samples were collected every 5 d, starting from day 0 (no exposure) to day 50. At the beginning (on days 0, 5, 10, and 15), six mussels were removed from the tanks (two mussels from each tank) each time for examination. On days 20 and 25, only five mussels were used each time (two mussels from each of the first two tanks and one mussel from the third). In the late stage (on days 30, 35, 40, 45, and 50), only three mussels were used each time (two mussels from the first tank and one mussel from the second).

Experiments were conducted to investigate the biokinetics of cesium in four parts of *Perna viridis,* namely, gill, viscera, adductor muscle, and foot. Thus, a small amount of a homogenized tissue from each of these four parts were taken out: gill (2–3 g wet weight), viscera (4–6 g wet weight), adductor

Table 1. Average concentrations of Cs in water (μg/ml) and different compartments of *Perna viridis* (μg/g) (dry mass) at different culture times[a]

Culture time (d)	Water	Gill	Viscera	Adductor muscle	Foot
5	7.04 ± 0.25	456 ± 299	349 ± 103	307 ± 224	298 ± 161
10	8.29 ± 1.64	468 ± 72	434 ± 103	259 ± 103	367 ± 136
15	8.45 ± 1.20	782 ± 163	766 ± 177	574 ± 183	833 ± 158
20	7.72 ± 0.85	735 ± 133	822 ± 220	620 ± 296	817 ± 80
25	9.56 ± 2.34	809 ± 58	808 ± 232	699 ± 211	850 ± 189
30	9.68 ± 1.04	676 ± 34	664 ± 97	1,020 ± 170	851 ± 58
35	10.1 ± 1.2	643 ± 135	820 ± 116	1,100 ± 150	959 ± 154
40	9.35 ± 0.55	707 ± 102	871 ± 169	1,030 ± 130	872 ± 145
45	8.93 ± 0.83	790 ± 187	675 ± 170	1,010 ± 100	1,010 ± 110
50	9.21 ± 0.26	697 ± 126	617 ± 137	959 ± 118	852 ± 107

[a] The ± values represent 1 SD.

muscle (0.6–0.9 g wet weight), and foot (0.3–0.6 g wet weight). These were then oven-dried at 70°C to constant weights. A tissue sample was then immersed into concentrated nitric acid (16 M) for acid digestion at 160°C until a clear solution was obtained. The sample was then filtered and adjusted to 10 ml with distilled water before the Cs determination. At the same stipulated culture time, 10 ml of water were drawn from each tank to determine the actual Cs concentrations in water.

Stock solutions used in the dosing/exposure experiment were prepared by dissolving CsCl (99.999% purity, Amersham Pharmacia Biotech, Piscataway, NJ, USA) in natural seawater. The total Cs concentration of each tissue sample was measured once using flame atomic absorption spectrophotometry (Shimadzu AA-6501S, Shimadzu Scientific Instruments, Columbia, MD, USA) based on the direct air–acetylene flame method described in [12]. The conditions used were as follows: atomic absorption mode, $\lambda = 852.1$ nm; current = 16; bandwidth = 1.0 nm; fuel = 1.8 L/min air–C_2H_2; burner's height = 7 mm. All concentrations are expressed in μg/g oven-dry-weight basis. Stock solutions used in the Cs calibration were prepared by dissolving CsCl (Amersham Pharmacia Biotech) in distilled water. Concentrations used for the calibration curve were 0, 1, 2, 5, and 7 μg/g. Samples with Cs concentrations exceeding this range were appropriately diluted prior to determination by atomic absorption spectrophotometry. All reagents used in this analysis were of analytical grade (Riedel-deHaën, Seelze, Germany). Oyster tissue of known background Cs level was spiked with 1, 10, and 100 μg/g Cs and analyzed as described previously to validate the analytical method used in this study. Recoveries ranged from 96 to 106% ($n = 12$). The coefficients of variation on replicate, spiked samples were all less than 6%.

The concentrations of Cs in water (μg/ml) and different compartments of *Perna viridis* (μg/g) at different culture time are shown in Table 1.

MODEL

From Table 1, the concentration q_w (in μg/ml) of Cs in water was observed to increase with time t (in days) in the form of $q_w = A - Be^{-jt}$, for which $A = 9.385$, $B = 4.385$, and $j = 0.1146$ were found from the best fit (Fig. 1). The time variation of the Cs concentration in the tanks was complex, which was affected by the attachment of Cs to the inner surfaces of the tank and by the decrease in the volume of seawater in the tanks. The observed increase in the concentration in water was due to the dominant decrease in the volume of seawater in the tanks that in turn was due to evaporation, the latter being enhanced by air conditioning to maintain a constant

temperature and also the continuous aeration of the seawater. The variation in the concentration also showed the importance of experimental determination of the Cs concentration in water at the same stipulated culture time of the mussel. Although Cs concentrations in water were not maintained at a constant level over the exposure period, availability of the actual Cs concentrations at each sampling intervals still allowed a meaningful modeling of the mussel biokinetics.

A wide choice of mathematical models are available to quantitatively describe the transfer mechanisms of the radionuclides from contaminated water into the mussel and between different compartments of the mussel, as many of studies of modeling techniques have been carried out [13–15]. Nevertheless, it is believed that the simplest model that can reflect the principal transfer characteristics should be adopted.

In the present work, the gill, viscera, adductor muscle, and foot of *Perna viridis* were modeled by four compartments and the biokinetics modeled by first-order linear differential equations. The compartment scheme for the four compartments are represented in Figure 2. Parameters used in the compartment scheme ($i = $ G,V,A,F stands for the compartments of gill, viscera, adductor muscle, and foot, respectively) are listed in Table 2. The differential equations governing the biokinetics and the time-dependent Cs concentrations in each of the four compartments are as follows:
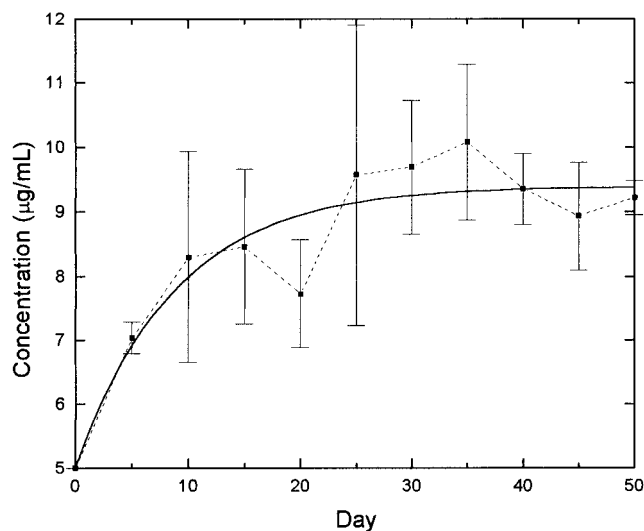

Fig. 1. Concentration (μg/ml) of Cs in water at different times. Solid line: best fit. The error bars represent 1 standard deviation.

Differential equations governing the biokinetics:

$$\frac{dq_G}{dt} = C_G \times (A - Be^{-jt}) - k_1 q_G \tag{1}$$

$$\frac{dq_V}{dt} = \frac{m_G}{m_V} k_1' q_G - k_2 q_V \tag{2}$$

$$\frac{dq_A}{dt} = \frac{m_V}{m_A} f_1 k_2' q_V - \lambda_A q_A \tag{3}$$

$$\frac{dq_F}{dt} = \frac{m_V}{m_F} f_2 k_2' q_V - \lambda_F q_F \tag{4}$$

Time-dependent Cs concentrations in each of the four compartments (general solutions with the initial conditions $q_G(0) = q_V(0) = q_A(0) = q_F(0) = 0$):

$$q_G = C_G \times \left[\frac{A}{k_1}(1 - u) - \frac{B}{k_1 - j}(x - u) \right] \tag{5}$$

$$q_V = C_V \times [L + (M + N - L)v - Mz - Nu] \tag{6}$$

$$q_A = C_A \times \left[\frac{L}{\lambda_A}(1 - x) + (M + N - L)\left(\frac{v - x}{\lambda_A - k_2}\right) \right.$$
$$\left. - M\left(\frac{z - x}{\lambda_A - j}\right) - N\left(\frac{u - x}{\lambda_A - k_1}\right) \right] \tag{7}$$

$$q_F = C_F \times \left[\frac{L}{\lambda_F}(1 - y) + (M + N - L)\left(\frac{v - y}{\lambda_F - k_2}\right) \right.$$
$$\left. - M\left(\frac{z - y}{\lambda_F - j}\right) - N\left(\frac{u - y}{\lambda_F - k_1}\right) \right] \tag{8}$$

where

$$k_1 = k_1' + \lambda_G; \qquad k_2 = k_2' + \lambda_V \tag{9}$$

$$u = e^{-k_1 t}; \qquad v = e^{-k_2 t}; \qquad x = e^{-\lambda_A t}; \qquad y = e^{-\lambda_F t};$$

$$z = e^{-jt} \tag{10}$$

$$C_G = \frac{C}{m_G}; \qquad C_V = k_1'\frac{C}{m_V}; \qquad C_A = f_1 k_1' k_2'\frac{C}{m_A};$$

$$C_F = f_2 k_1' k_2'\frac{C}{m_F} \tag{11}$$

$$L = \frac{A}{k_1 k_2}; \qquad M = \frac{B}{(k_1 - j)(k_2 - j)};$$

$$N = \frac{(A/k_1) - [B/(k_1 - j)]}{k_2 - k_1} \tag{12}$$

Thus, if k_1, k_2, λ_A, λ_F, C_G, C_V, C_A, and C_F are determined, the biokinetics of Cs in all the four compartments in *Perna viridis*, namely, gill, viscera, adductor muscle, and foot, can be known.

RESULTS AND DISCUSSION

The experimental data in Table 1 were fitted by Equations 5 to 12 using user-defined expressions in the nonlinear curve fit program of the Microcal™ Origin™ (Ver 5.0, Microcal Software, Northampton, MA, USA) with the interested parameters as the user-defined parameters. The Cs levels in all four compartments at day 0 were generally low and close to zero. The results are shown in Figures 3 to 6 and are summarized in the following:
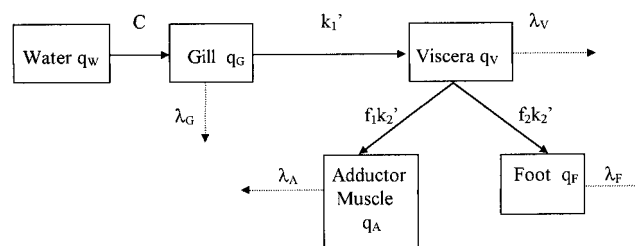
Compartment scheme:

Fig. 2. Compartment scheme for the four compartments of *Perna viridis*: gill, viscera, adductor muscle, and foot.

$C_G = 32.4$		(mL g^{-1} d^{-1})
$C_V = 17.2$		(mL g^{-1} d^{-2})
$C_A = 2.47$	$C_F = 7.34$	(mL g^{-1} d^{-3})
$k_1 = 0.410$	$k_2 = 0.510$	
$\lambda_A = 0.104$	$\lambda_F = 0.356$	(d^{-1})

A measure for the variation of the parameters calculated is not available using the Microcal Origin software.

The weights of different parts of *Perna viridis* were also measured as shown in Table 3. From the results of best fits and the measured weights of different parts of *Perna viridis,* the following were also determined:

$$f_1 = 0.47 \qquad f_2 = 0.53 \qquad k_2' = 0.045 \quad (d^{-1})$$

In this study, the soft tissues that remained after removal of the gill, adductor muscle, and foot were referred to as the viscera. It is interesting that a value of k_1' consistent with both k_1 and C_v could not be obtained if the viscera was treated as one single homogeneous compartment. The problem, however, could be solved if the viscera was represented by more than one compartment containing different Cs concentrations; here the simplest model, that is, a two-compartment model, was adopted for the viscera (see the compartment scheme of the viscera in Fig. 7).

Here compartment 1 is fast, whereas compartment 2 is slow (in terms of their elimination rates of Cs), so $q_2 \gg q_1$ and $q_V \gg q_1$. Effectively, Cs enters only the fast compartment in the viscera and is transferred only from this compartment in the viscera to the adductor muscle and the foot. In this way, C_V was not directly relevant in the model; instead, $C = (k_1')C/m_1$

Table 2. Parameters used in the compartment scheme shown in Figure 2[a]

Parameter	Description	Unit
C	(μg of Cs into gill/d) per (μg/ml of Cs in water)	ml/d
k_1'	Transfer coefficient from gill to viscera	d^{-1}
$f_1 k_2'$	Transfer coefficient from viscera to adductor muscle	d^{-1}
$f_2 k_2'$	Transfer coefficient from viscera to foot	d^{-1}
q_i	μg/g of Cs in the ith compartment	μg/g
m_i	Dry mass of the ith compartment	g
λ_i	Elimination rate from the ith compartment	d^{-1}

[a] i = G, V, A, F stands for the compartments of gill, viscera, adductor muscle, and foot, respectively.
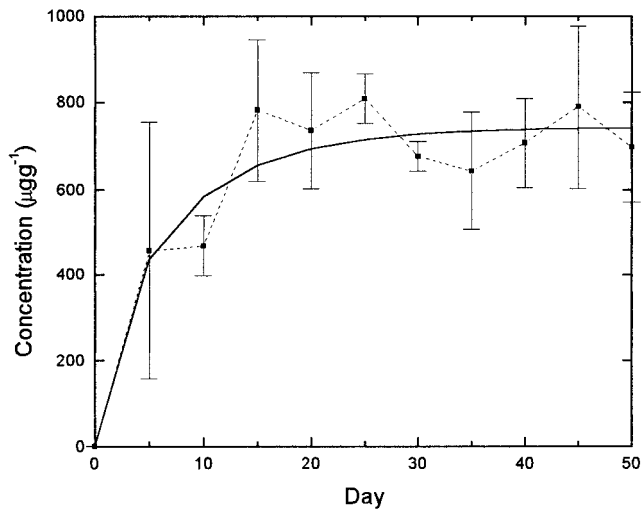
Fig. 3. Concentration (μg/g) of Cs at different culture times in the gill of *Perna viridis*. Solid line: best fit. The error bars represent 1 standard deviation.

Fig. 5. Concentration (μg/g) of Cs at different culture times in the adductor muscle of *Perna viridis*. Solid line: best fit. The error bars represent 1 standard deviation.

should be obtained from the best fit to data of q_1. When the experimentally measured values of q_V, which were much larger than q_1, were used instead, the derived k_1' would be too large. This explains why k_1' could not be found to be consistent with both k_1 and C_V. Nevertheless, the previously derived value of C_V could still be used for determination of concentration factors (see the following) because Equation 6 could now be treated as an empirical formula for calculation of the Cs concentration in the whole viscera.

Concentration factors (CFs) were derived from the previous results. The CFs for individual compartments (CF_i) and for *Perna viridis* (CF_P) were determined. In Till and Meyer [16], the CF is defined as the ratio between the equilibrium concentration in an organism (per kilogram fresh mass) and the concentration in water (per liter of water). This definition was adopted for CF in the present work, with the unit (L/kg or ml/g).

The CFs were inferred from the derived parameters as follows. For determination of the CFs, a constant input was adopted; that is, the activity concentration in water was A, $B = 0$,
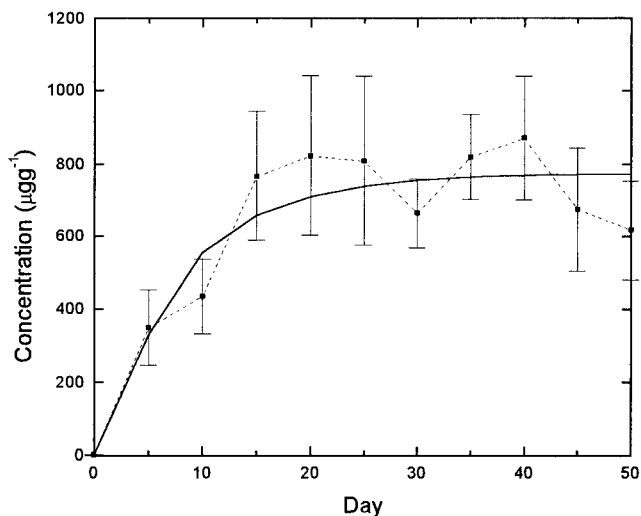
and $u = v = x = y = z = 0$, so when considering the dry–fresh ratios (R_i), we have

$$CF_G = \frac{C_G}{k_1} \times R_G, \qquad CF_V = \frac{C_V}{k_1 k_2} \times R_V,$$

$$CF_A = \frac{C_A}{k_1 k_2 \lambda_A} \times R_A, \qquad CF_F = \frac{C_F}{k_1 k_2 \lambda_F} \times R_F$$

$$CF_P = \sum_i (CF_i \times wetmass_i) \bigg/ \sum_i wetmass_i$$

Thus, for our data,

$$CF_G = 8, \qquad CF_V = 12, \qquad CF_A = 24, \qquad CF_F = 22,$$

$$CF_P = 12 \ (L \ kg^{-1})$$

Because the mass of viscera was much larger than those of other tissues, CF_P was close to CF_V.

When the biological half-life of a compartment was much

Fig. 4. Concentration (μg/g) of Cs at different culture times in the viscera of *Perna viridis*. Solid line: best fit. The error bars represent 1 standard deviation.
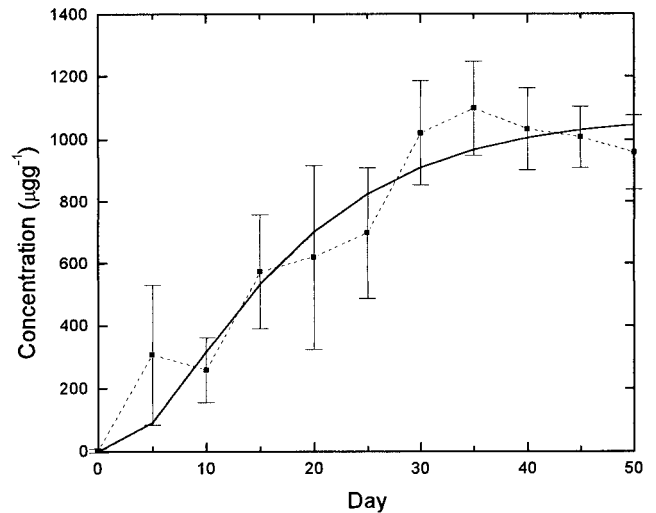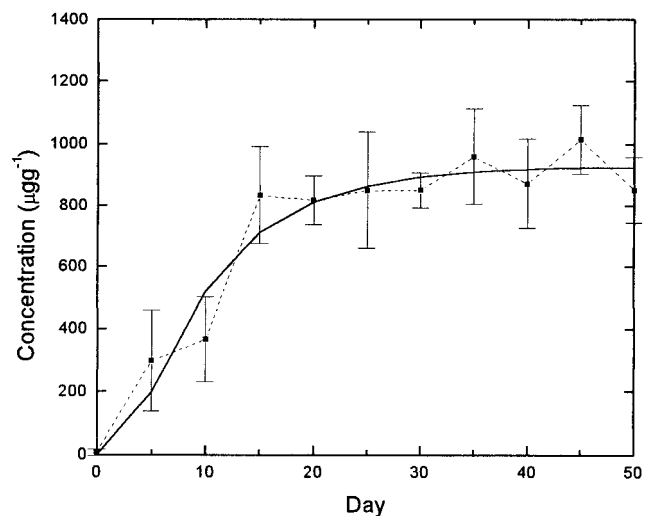

Fig. 6. Concentration (μg/g) of Cs at different culture times in the foot of *Perna viridis*. Solid line: best fit. The error bars represent 1 standard deviation.

Table 3. Measured weights of different parts of *Perna viridis*

	Adductor muscle	Gill	Foot	Viscera
Mean wet weight (g)	0.67 ± 0.18	2.16 ± 0.52	0.25 ± 0.16	6.72 ± 0.61
Converted dry weight (g)	0.14	0.22	0.055	0.97

smaller than the physical half-life of radioactive cesium (30.1 years for ^{137}Cs) or the elimination rate of a compartment was much larger than the physical decay rate of radioactive cesium, both of which were true for all the considered compartments of *Perna viridis,* the CFs for stable Cs were effectively the same as those for radioactive cesium [16]. For comparison, the CF reported for Mussels was 15 [17], and the range summarized for mollusks was 9 to 50 [16]. It can be seen that the CF value determined for *Perna viridis* in the present work agrees well with the reported values.

It is worth noting that although the current experiments relied on measurement of stable element concentrations, the present investigation should still be useful in providing prediction of the radioactive cesium activity in each compartment of *Perna viridis,* given that the biokinetics of these elements should be a function of their chemical properties only [16]. The concentration factor approach used in the previous analysis, which is an established methodology and an important component of radioecological studies [18], presumes that the concentration of an element in an aquatic organism is directly proportional to the concentration of that element in water [16]. In this way, although the Cs concentrations employed in the present experiments were higher than those expected in the environment, the CFs thus determined should reflect the true values. Furthermore, the consistency between the CFs obtained in the present work and the previous results in the literature obtained using environmental levels of radioactive cesium also demonstrated the feasibility of the concentration factor approach in this type of investigations.

Cesium is a well-known analogue of potassium and was reported to be accumulated by animals by substitution during uptake processes [19]. The agreement of the experimental data with the asymptotic behavior in attaining equilibrium Cs concentrations in the investigated compartments of *Perna viridis* were in support of this mode of accumulation.
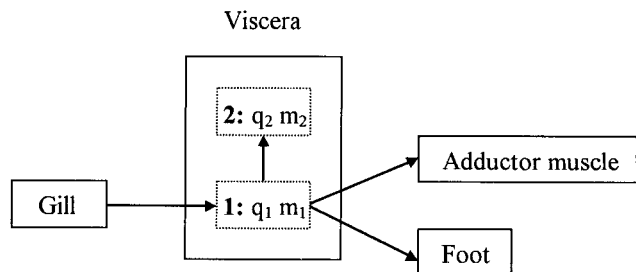
Fig. 7. The compartment scheme in which the viscera was represented by a two-compartment model.

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