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Radionuclide Accumulation by Anodonta piscinalis Nilsson (Lamellibranchiata) in a Continuous Flow System*)

by

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(with 3 figs.)

Introduction

Freshwater species of the phylum Mollusca have for a long time been recognized as useful indicators for radioactive contamination of inland recipients. But the knowledge about their ability to concentrate specific radionuclides is based on limited experimental investigations only. The information available has come from a number of sources, including studies of contaminated water courses – Nelson (1962), environmental measurements of natural and fall-out radioactivity – Ravera et al. (1961), and aquaria experiments under laboratory conditions – Polikarpov (1960). The laboratory investigations have been performed with a few species only, and a very restricted selection of radionuclides.

It was considered of interest to study the accumulation of radionuclides by a freshwater lamellibranch under conditions such that the environmental factors and the organism interacted in approximately the same way as in nature.

During an experimental investigation on bioaccumulation and transfer of some radionuclides among a few components of freshwater communities, we had the opportunity to do a field experiment on the

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response of *Anodonta piscinalis* NILSSON to six radionuclides. Specimens of *Anodonta piscinalis* were exposed for 125 days to approximately constant concentrations of the radionuclides phosphorus 32, cesium 137, strontium 89, cerium 144 and zirconium 95 – niobium 95. In addition, ruthenium 103 was introduced into the system during the last 55 days of the experimental period.

A model recipient through which flowed water pumped from a nearby river, provided a biotope for *Anodonta piscinalis* with the following properties: Water of the same chemical quality and with mainly the same temperature variations as that in the river, a natural food supply for the test animals and exposure to selected radionuclides at controlled concentrations. Thus it was possible to describe the bioaccumulation of the radionuclides together with the conditions under which it occurred.

THE GENERAL PROBLEM

The ratio of the concentration of an element in the organism to the concentration in the environment is an expression of the possible accumulation factor for the radioactive element. Our knowledge of the elementary composition of molluscs is unfortunately very slight at the present time. VINOGRADOV states that the analyses are less complete than for other groups of organisms (1953, p. 271). Quantitative data are almost absent for lamellibranchs, and the scanty information found in literature is rather old. Modern investigations of the mineral metabolism of lamellibranchs have centered around physiological aspects of osmoregulation and shell formation (for references see MORTON 1958, JODREY et al. 1955, KADO 1960).

It is known that certain elements are found in remarkably high concentrations in *Anodonta*. This applies to phosphorus – KADO (1960, p. 172), manganese – VINOGRADOV (1953, p. 325) and the materials of which their valves are constructed. Almost nothing is known about the mineral composition in relation to individual development, environmental conditions and seasonal variation.

Species of the genus *Anodonta* are ciliary feeders and live on suspensions or deposits. The food is taken from the particle fraction of the surrounding water without selection or discrimination. Filtering is very efficient, and it is reported that seston down to 1 μ in size can be retained by their filtering organs – MORTON (1958, p. 77). The animal is permeable to water and excretes a dilute urine. A high daily water flux through the body has been demonstrated experimentally. There are varying estimates in the literature of the daily quantity of urine excreted. PROSSER et al. (1961, p. 25) state that the urine amounts to 50% of the body volume per day. PICKEN (1937, p. 28) showed that with the pericardium experimentally opened *Anodonta cygnea* at 18°C excreted approximately six times its own weight of water in one day. The last mentioned author calculated that the weight of salt ingested in the form of algae in one day was approximately $1.25 \, . \, 10^{-2}$ g, the algal matter being removed from 214 liters of water by filtration.

The valves of lamellibranchs are composed of about 99% $CaCO_3$. Of the three distinct layers of the shell, the periostracum is organic in nature and has a protective function. The building materials for the valves are taken both from particulate food and from dissolved matter in the water. Recent physiological investigations using Ca^{45} and Sr^{90} show that, while these elements are taken up rapidly from the gut and distributed by the blood, they may also be taken directly from the water by the mantle cells and used for shell formation – BEVELANDER (1952), FRETTER (1953), RAO et al. (1954), and KADO (1960).

These, and other ecological and physiological characteristics of freshwater lamellibranchs have drawn attention to their possible use as effective indicators of radioactive contamination in recipients of wastes from atomic energy establishments – BERG et al. (1961), NELSON (1962). The lamellibranchs are sedentary members of the bottom community, and, belonging to the group of organisms known as 'integrating water samplers' – NELSON (1962) – they are regarded as environmental monitors which give a good deal of information about the contamination of the recipient for a minimum amount of effort. Transitory situations existing in the environment should be reflected in the radioactivity of the soft tissues. The valves, on the other hand, should show a level of radioactivity which is the result of an accumulation over a longer period of time. Both aspects are important in monitoring programmes of contaminated aquatic environments.

EXPERIMENTAL PROCEDURE

Introductory work with *Anodonta piscinalis* was carried out during the summer of 1961. Determinations of the uptake of radiophosphorus were made at intervals during ten days of continuous exposure to the isotope. In addition, short time exposure with several radionuclides was tried. The experience gained from this work was used to advantage in the experiments here described, which were performed in the period June 25th – October 29th, 1962.

The model recipient

The experiment was conducted in a model recipient. Two channels made of cement-asbestos, each 120 m long and with a cross-section of 0.03 m², were continuously charged with river water at one end (0.5 ± 0.03 /sec.). A discharge weir at the other end of the channels kept the water depth at 0.10 m. A layer of pebbles was placed on the bottom of each channel, with a thickness of about 3 cm. The model recipient has been operated during the summer season (May—October) for two successive years. A complete description of the model recipient and its function will be given in a separate paper (GARDER et al., in preparation).

The environmental conditions

The river from which the water used for the experiment was taken, has its drainage area in a landscape of gneissic and plutonic rocks covered chiefly with glacifluvial and marine deposits of sand and clay.

TABLE I

Chemical and biological characteristics of the water used for the experiment.

Chemical data:				Biological data:
Component	Maximum value	Minimum value	Arithmetical means	Dominant species in the communities
pH	7.6	6.4	7.1	*Vegetation:* *Fragilaria capucina* DESMAZIE *Melosira varians* C. A. AG. *Oedogonium* LINK sp. (43 *Oscillatoria* cf. *brevis* (KÜTZ GOM. *Spirogyra* cf. *formosa* (TRANS CZURDA. *Spirogyra* cf. *porticalis* (MÜLLER) CLEVE. *Vaucheria walzii* ROTHERT. *Fauna:* *Carchaesium polypinum* L. *Centropilum luteolum* (MÜLLER *Chironomus thummi* K. *Eurycercus lamellatus* (O. F. MÜLLER). *Lymnaea pereger* MÜLLER. *Philodina* EHRENBERG sp. *Planorbis planorbis* L. *Plumatella fruticosa* ALLMAN.
$\varkappa 20 (n.10^{-6})$	103.5	41.3	57.2	
$KMnO_4$, mg O_2/l	6.1	3.9	5.2	
Ca, mg/l	6.1	4.8	5.4	
Mg, mg/l	1.5	0.7	1.1	
Fe, mg/l	0.5	0.1	0.2	
Cl, mg/l	9.0	2.1	4.8	
SO_4, mg/l	9.0	7.0	7.9	
Na, mg/l	4.0	0.8	2.1	
K, mg/l	1.5	0.7	1.0	

The water is somewhat polluted by drainage water from cultivated land and by sewage from populated areas. The chemical composition of the water is given in Table I. The data listed in the table represent the results of measurements carried out at monthly intervals during two years. Daily observations of the components involved demonstrated that short time variations were of considerable magnitude.

The water temperature was measured at daily intervals during the long term exposure of *Anodonta piscinalis*. The average temperature values are given in Table II.

TABLE II

Water temperature data:

Period of 1962	°C
June 25—August 6	17
August 6—September 12	13
September 12—October 10	10
October 10—October 29	5

A layer of clayish sediments was formed in the channels after a few months of operation. The sediment layer partly covered the pebbles, especially in the first sections of the channels. The mineral component of this bottom deposit was chiefly pleistocene marine clay transported by the river water from the catchment area.

Communities of river biota (see Table I)established themselves in the throughflow channels during the first summer of the operation. Filamentous green algae together with a diverse diatom flora were distinctive for the vegetation. The fauna was dominated by species of crustaceans and insects. The communities were initiated by organisms and diaspores transported with the river water. No organisms were transplanted to the channel biotope except for the lamellibranchs used for the experiment.

The selected specimens of *Anodonta piscinalis* were introduced into the last two sections of 2 m length at the end of each channel. According to their sedentary nature, the test animals here partially embedded themselves in the mud between the pebbles, and showed only a limited tendency to locomotion inside the boundaries of their environment. Fig. 1 shows a section of a throughflow channel with a specimen of the test animal. The anterior two-thirds of the animal lie buried in the sediments between the pebbles at the bottom of the channel. The dark areas with vegetation are algal communities dominated by *Spirogyra* cf. *porticalis*.

The species used in the experiment was *Anodonta piscinalis* NILS-

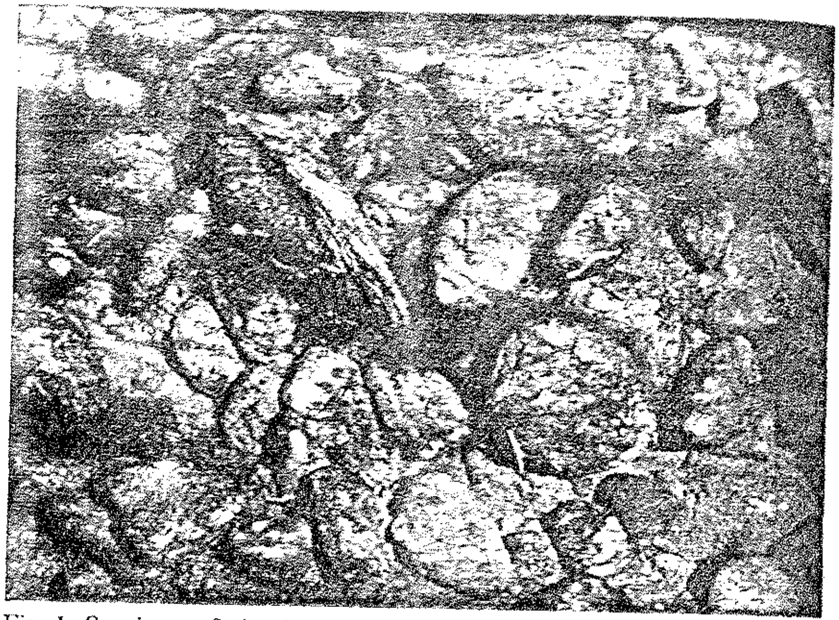

Fig. 1. Specimen of *Anodonta piscinalis* in the channel environment during the experiment.

SON of the family Unionidae, order Eulamellibranchiata of the bivalve Mollusca. The diagnosis was verified by JAN ØKLAND at the Zoological Laboratory of the University of Oslo. *Anodonta piscinalis* NILSSON corresponds to *Anodonta cygnea piscinalis* NILSSON, EHRMANN (1933) and *Anodonta cygnea anatina* (L.) (= *Anodonta piscinalis* NILSSON), MANDAHL – BARTH (1949). The intricate taxonomy of the species of the family Unionidae is reflected in the diverse synonyms found in the literature.

A recent investigation – ØKLAND (1963), has furnished knowledge on the population density, age distribution, growth and habitat of *Anodonta piscinalis* NILSSON in a eutrophic lake in southeastern Norway.

Freshwater lamellibranchs previously used in experiments on bioaccumulation of radionuclides include *Unio pictorum* L., BERG et al. (1961) and *Anodonta cygnea* L., TIMOFEEV – RESOVSKII et al. (1961).

The specimens used for the present investigation were collected from biotopes in a similar chemical milieu to that of the water used for the experiment. All the test animals were of about the same size and stage of growth. The animals were in healthy condition throughout the whole of the experiment. The circumstances to which they were exposed for such a long period were apparently not harmful to their growth or wellbeing.

Weight relations of typical test animals are presented in Table III. Thirty-five specimens of the test animals were used for the determinations of dry weight and ash (for details, see p. 159). The result is given in Table IV. Wet weight determinations of molluscs are subject to uncertainty, the main error involved being the varying amount of water and body fluid in the organism.

TABLE III

Wet weight relations of typical test animals. The values indicate g.

Wet weight of test animal	Valves	Body	Valves in per cent of whole animal
25.59	9.56	16.03	37.3
26.24	9.00	17.24	34.4
28.30	11.14	17.16	39.4
29.01	11.09	17.92	38.4
29.68	12.35	17.33	41.7
29.76	9.77	19.99	32.8
29.82	12.00	17.82	40.2
30.96	10.15	20.81	32.7
31.13	12.58	18.55	45.6
32.21	8.69	23.52	27.0
34.12	13.46	20.66	39.2
34.83	13.14	21.69	37.8
35.94	15.11	20.83	42.0
37.76	16.35	21.41	46.0
42.58	14.26	28.32	33.5

TABLE IV

Dry weight and ash of body and valves. Average of 35 *specimens.*

Part of animal	Dry weight in per cent of wet weight	Ash in per cent of wet weight	Ash in per cent of dry weight
Body	12	2.1	18
Valves	95	90	95

The radionuclides, radiochemical methods and procedures

The radionuclides used in the experiments are listed in Table V. Except for P^{32}, all radionuclides are important fission products, with a long half life and high fission yield. P^{32} was chosen because of the biological importance of this element.

The radionuclides Zr^{95} and Nb^{95} were in equilibrium in the added solution, and no separation of the two isotopes was performed.

TABLE V

Physical and chemical characteristics of the radionuclides.

Isotope	Half life	Specific activity	Chemical state
P^{32}	14.2 days	100 mC/mg P	Ortho-phosphate
Sr^{89}	51 days	carrier free	Sr-chloride
Cs^{137}	30 years	25 mC/mg Cs	Cs-chloride
Ru^{103}	40 days	1 mC/mg Ru	Ru-trichloride
Ce^{144}	285 days	carrier free	Ce-chloride
Zr^{95}/Nb^{95}	65 days/35 days	carrier free	Oxalate-complex

P^{32}, Ru^{103} and Zr^{95}/Nb^{95} were dosed to one throughflow channel and Sr^{89}, Ce^{144} and Cs^{137} to the other. Except for Ru^{103} the radioisotopes were added continuously to the channels from June 25th up to the 105th day of the experiment, when the dosing was terminated. Dosing of Ru^{103} commenced on September 4th, the 72nd day of the experimental period.

Fresh solutions of the radionuclides were made up twice a week, and the amounts of activity added to the channels were calculated to give final concentrations of the radionuclides as indicated in Table VI.

The dosing of the radionuclides was routinely controlled and adjusted if necessary twice a day. Daily water samples were collected in the channels and preserved with formalin. Mixed samples representative for one week were prepared and used for radiochemical analysis of the appropriate radioelements. The results of the measurements in these weekly controls are listed in Table VI. The figures indicate the total content of radioactivity per ml of mixed water sample. There was a difference between the theoretical figures and the measured values for the concentrations in the channel water. This may be explained by the behaviour of the radionuclides in the channel environment and in part by the storage of the water samples. The affinity of the different radionuclides to the biota is of particular importance, since it was hardly possible to get water samples from the channels without including organisms, detritus and bottom deposits.

The concentrations of radionuclides in the channels were extremely small, so that the solubility products were not significantly affected by the addition; and the radionuclides did not precipitate.

TABLE VI

Concentrations in the channel water after addition of the radionuclides. The values indicate 10^{-6} μc/ml.

Radionuclide	Theoretical concentration	Concentrations measured in the weekly controls
P^{32}	2	5.8 ± 0.5
Sr^{89}	2	7.8 ± 1.1
Cs^{137}	2	2.1 ± 0.3
Ce^{144}	6	6.0 ± 1.0
Zr^{95}/Nb^{95}	2	1.2 ± 0.2
Ru^{103}	4	7.8 ± 1.5

The test animals collected for measurements of radioactivity were first carefully cleaned of surface impurities by rubbing and washing with water, and then dissected and the body and valves separated. The muscles shutting the valves were cut with a scalpel introduced between the hinge line. The mantle was cut away from its line of attachment, and the soft parts were removed. The valves and the body were immersed in water from the respective channel and transported to the laboratory for radiochemical analysis. The two sections were weighed, dried at 105°C and then ashed at 450°C for 24 hours.

Ru^{103} and Zr^{95}/Nb^{95} were determined by gamma-spectrometric methods. P^{32} was determined by beta-counting, subtracting the beta-activity from Ru^{103} and Zr^{95}/Nb^{95}.

In the same way Cs^{137} and Ce^{144} were determined by gamma-spectrometric methods, and Sr^{89} by beta-counting, subtracting the beta-activity from Cs^{137} and Ce^{144}.

The counting procedure was standardized by adding known amounts of the radionuclides to inactive samples and counting.

Because of the very low activity in a few samples, Sr^{89} and P^{32} were determined by the conventional radiochemical procedures recommended by the WORLD HEALTH ORGANIZATION (1959).

Two animals were taken from each channel during the first three day period of sampling. The double set of figures in Table VII, concerning these days, represents the result of the radiochemical analysis for each element. The scattering of the results was small, with few exceptions, and subsequent determinations were restricted only to one animal from each channel on every day of sampling.

During the experimental period a control channel was operated without dosing of radionuclides. Determinations of background radioactivity as total beta radiation were carried out at intervals. Specimens of *Anodonta piscinalis* collected from this channel and subjected to radioactivity measurements, demonstrated that the background radioactivity varied from 0.1—4.2 . 10^{-7} μc/mg ash weight for the body and from 0—3.6 . 10^{-7} μc/mg ash weight for the valves. Variations in background radioactivity were therefore of negligible significance for the results obtained in the contaminated channels.

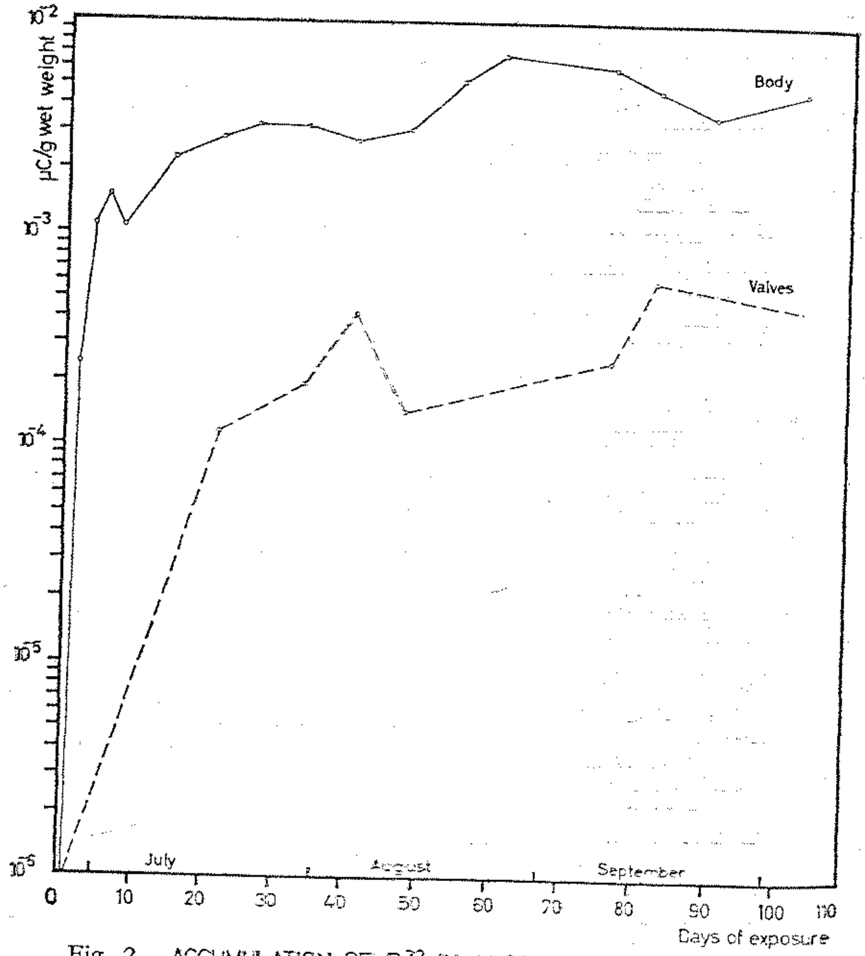

Fig. 2. ACCUMULATION OF P^{32} IN ANODONTA PISCINALIS

RESULTS

Uptake is defined as the entry of ions into cells, tissues or organs by any mechanism including adsorption. The net result of the processes of uptake and loss of ions by the organism or its different parts at a given moment is in the following designated as accumulation.

The accumulation of the six radioelements by *Anodonta piscinalis* during the experimental period is listed in Tables VII and VIII.

After one month's exposure the animals had reached a level of radioactivity both in body and valves which was maintained, with slight fluctuations, during the rest of the experimental period. The results concerning the individual radionuclides are commented below.

Phosphorus – 32.

The data obtained are plotted in fig. 2. The level of radionuclide concentration fluctuated rapidly both in the body and the valves during the first days of exposure. Subsequently, a slow increase of radioactivity in the two fractions was observed, and a steady state was reached after 20—30 days of exposure. The pattern of accumulation during the remaining period was characterized by some fluctuations. High values observed during August—September coincided with high levels of radioactivity from the other radionuclides.

After the termination of radionuclide addition on the 105th day of exposure, the level of P^{32} remained high until the last days of observation, and no loss of the radioelement from the test animals was observed.

The maximum concentration of P^{32} measured corresponds to $5855 . 10^{-4}$ μc/g ash weight of the body, and $5.6 . 10^{-4}$ μc/g ash weight of the valves.

Strontium – 89

The data obtained are presented diagrammatically in fig. 3. The curves for the accumulation in the body and the valves lie closer together for this element than for P^{32}. Fluctuations of the accumulation level did occur also after the first 30 days of exposure. The high figures for the valves on the 34th and 76th day of exposure are questionable and should be disregarded.

The maximum concentration of the radioelement measured corresponds to $933 . 10^{-4}$ μc/g ash weight of the body, and $7.0 . 10^{-4}$ μc/g ash weight of the valves.

Cesium – 137.

With reference to the figures presented in Tables VII and VIII it is seen that the accumulation of this radioelement reached a uniform level more rapidly in the body than in the valves. The concentration of the radioelement on a wet weight basis is at times higher in the valves than in the body. In both fractions the highest values for the accumulation were measured in the period from the 76th to the 82nd day of the exposure.

The maximum concentration of the radioelement corresponds to

TABLE VII

Accumulation of radionuclides in the body of Anodonta piscinalis. Each value refers to the analysis of one specimen.

1962	Days of exposure	Radioactivity μc. 10^{-4} per g wet weight					
		P^{32}	Sr^{89}	Cs^{137}	Ce^{144}	Zr^{95}/Nb^{95}	Ru^{103}
June	2	2.6	0.95	0.64	2.2	0.24	
	2	2.4	1.9	0.71	2.4	0.30	
	4	10.7	0.79	2.5	7.2	1.0	
	4	11.4	0.66	2.8	7.9	1.0	
July	6	12.4	2.3	0.95	1.6	0.21	
	6	15.1	1.2	1.05	2.9	0.15	
	8	10.9	3.8	1.9	5.0	0.31	
	15	22.4	2.4	3.1	10.8	0.80	
	22	28.5	4.1	3.0	7.7	1.2	
	27	32.7	4.6	3.6	3.6	1.2	
	34	32.1	7.7	2.9	6.0	0.78	
August	41	27.7	5.9	2.0	2.4	0.60	
	48	31.5	6.6	2.0	2.1	0.65	
	55	52.9	12.0	3.1	5.5	0.88	
	61	71.4	12.0	3.2	4.8	3.2	
September	76	62.1	19.6	2.4	14.7	1.9	5.7²)
	82	48.7	11.5	5.9	16.8	1.3	4.1
	90	36.9	8.7	4.6	10.0	1.4	5.9
October	103¹)	46.7	9.6	2.1	6.4	1.3	6.0
	107	91.6	2.7	1.0	4.2	1.2	7.0
	110	98.7	6.4	2.3	4.0	0.84	5.0
	114	68.5	4.4	1.1	2.5	0.55	4.2
	118	123.0	6.5	1.8	3.3	0.57	5.7
	124	81.2	6.5	1.0	2.8	0.45	3.3

¹) The addition of radionuclides was terminated on the 105th day of the experiment.

²) The 4th day with dosing of Ru^{103}.

$281 . 10^{-4}$ μc/g ash weight of the body, and $9.5 . 10^{-4}$ μc/g ash weight of the valves.

Cerium – 144.

The accumulation of this element is comparable to that of cesium – 137. Fluctuations of the level in the valves were only slight when steady state conditions had been established. The accumulation level in the body was highest from the 76th to the 90th day of exposure.

TABLE VIII

Accumulation of radionuclides in the valves of Anodonta piscinalis. Each value refers to the analysis of one specimen.

1962	Days of exposure	Radioactivity μc. 10^{-4} per g wet weight					
		P^{32}	Sr^{89}	Cs^{137}	Ce^{144}	Zr^{95}/Nb^{95}	Ru^{103}
June	2	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	
	4	—	—	—	—	—	
July	6	—	—	—	—	—	
	8	—	—	—	—	—	
	15	—	4.9	—	—	—	
	22	1.2	5.0	1.8	5.1	0.4	
	27	—	1.6	1.5	8.1	1.8	
	34	2.0	11.1	0.9	—	—	
August	41	4.3	3.3	0.9	—	—	
	48	1.5	4.6	1.6	—	1.2	
	55	—	6.4	—	—	—	
	61	—	2.9	—	—	—	
September	76	2.6	13.8	8.6	5.1	1.8	< 1[2]
	82	5.1	2.7	5.5	—	—	< 1
	90	—	1.9	3.7	—	3.1	< 1
October	103[1]	—	3.3	7.3	9.6	2.5	< 1
	107	—	2.5	4.2	—	1.5	< 1
	110	1.9	3.2	8.4	—	1.4	< 1
	114	—	1.3	6.3	—	1.2	< 1
	118	3.8	—	—	—	0.4	< 1
	124	—	—	3.1	3.9	—	< 1

[1] The addition of radionuclides was terminated on the 105th day of the experiment.

[2] The 4th day with dosing of Ru^{103}.

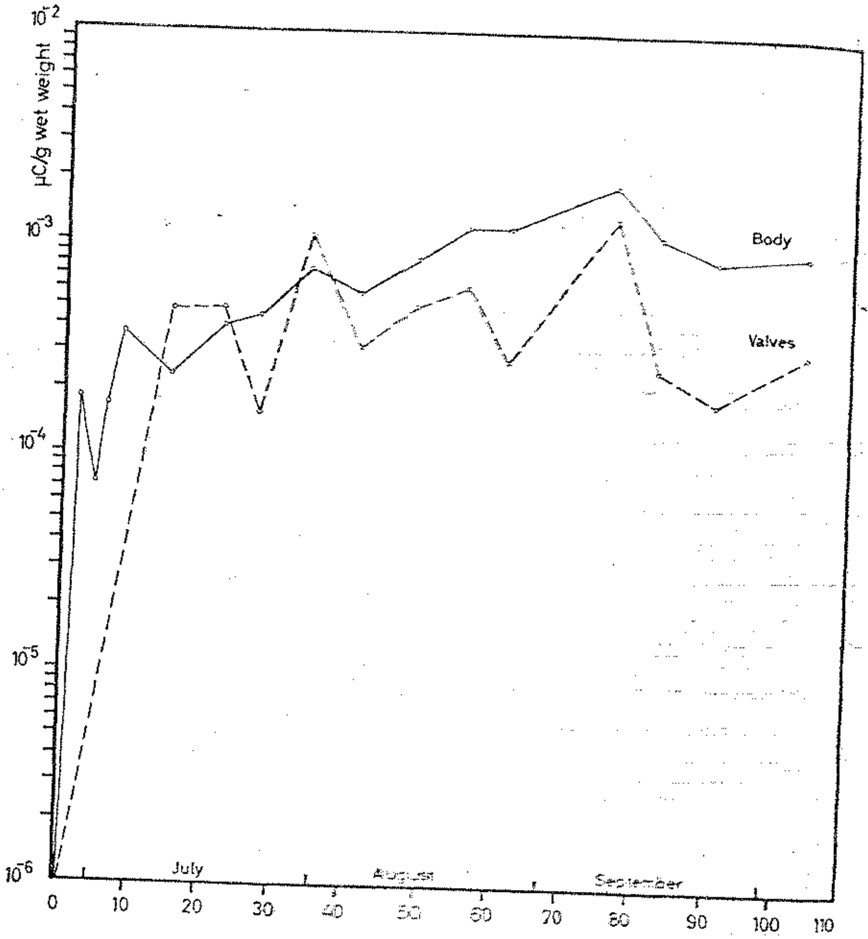

Fig. 3. ACCUMULATION OF Sr^{89} IN ANODONTA PISCINALIS

The maximum concentration of the radioelement measured corresponds to $800 \cdot 10^{-4}$ $\mu c/g$ ash weight of the body, and $10.6 \cdot 10^{-4}$ $\mu c/g$ ash weight of the valves.

Zirconium – 95, niobium – 95.

The accumulation of these radioelements was small.

The maximum concentration measured corresponds to $142 \cdot 10^{-4}$ $\mu c/g$ ash weight of the body, and $3.4 \cdot 10^{-4}$ $\mu c/g$ ash weight of the valves.

Ruthenium – 103.

The first observations were made on the 76th day of the experiment, i.e. the 4th day after the dosing of Ru^{103} was started. The accumulation of this element differed markedly from that of the other radionuclides investigated. The concentration in the valves was so minute that the radiochemical method applied gave negative results. The accumulation level in the body was more or less constant during the entire period of exposure.

The maximum concentration measured corresponds to $333 \,.\, 10^{-4}$ μc/g ash weight of the body.

Mean values for the accumulation level of the radionuclides (except for Ru^{103}) during the period of steady state conditions are given in Table IX. The figures are calculated on basis of the whole animal, using data from the 34th day of exposure to the end of the radionuclide addition.

TABLE IX

Accumulation level during steady state conditions. Values for radioactivity indicate μc. 10^{-4} per gram wet weight of the whole animal.

Isotope	Number of observations	Mean value	Standard deviation
P^{32}	9	29.8	3.1
Sr^{89}	9	8.0	0.8
Cs^{137}	9	3.5	0.3
Ce^{144}	9	7.5	1.1
Zr^{95}/Nb^{95}	9	1.6	0.2

DISCUSSION

The response of *Anodonta piscinalis* to prolonged exposure to approximately constant concentrations of radionuclides was an accumulation of the radionuclides both in the body and the valves of the test animal. The course of accumulation may be divided into two phases: Firstly, a steady increase of radioactivity over several days, and secondly, a steady state condition with respect to uptake and loss of the radionuclide resulting in a level of fluctuating accumulation with seasonal and physiological circumstances. The ratios of internal to external concentrations of particular radionuclides are variable, and can be understood only when considered in relation to the complicated series of factors on which they depend.

The present investigation indicates that there are marked variations of radionuclide accumulation in the body of *Anodonta piscinalis* depending upon the time of sampling. Most probably these fluctuations are the results of seasonal changes in the environmental conditions and periodic alterations in metabolism related to the development and growth of the test animals. The three radionuclides with the highest affinity for the body of the animal —P^{32}, Sr^{89} and Ce^{144}) showed maximum accumulation during the last days of August and the moth of September. No direct correlation with important milieu factors in the water was ascertained. Possible variations due to season have to be kept in mind when measurements of radioactivity in the body of lamellibranchs are used for the evaluation of recipient contamination.

The valves of freshwater lamellibranchs have been claimed to have the properties required of an indicator of long term contamination with radionuclides. Particularly should they be excellent organisms for studying the long term contamination of recipients with Sr^{90}. According to NELSON (1962, p. 38): 'The shell is deposited in distinct annual layers which are not subject to subsequent metabolism: consequently, the shell represents a history of the deposition of strontium'.

However, the levels of accumulation in the valves of *Anodonta piscinalis* were found to vary during the 125 days exposure of the animals to the concentrations of radionuclides indicated. This observation gives occasion to reflect upon the use of valves of freshwater lamellibranchs as long term indicators of radionuclides in contaminated watercourses.

Although the metabolic turnover of elements in the valves of freshwater lamellibranchs may be slow, it must be taken into consideration when the valves are intended for use in the description of long term contamination. The minerals in the valves may constitute a store of elements which can be depleted under exceptional physiological or environmental conditions.

A parallel example is the mobilization of minerals deposited in the bones of vertebrates. With regard to molluscs WAELE (1929) concludes that the formation of valves by *Anodonta cygnea* is a reversible process. When the test animal lived on a diet poor in calcium, this element was drawn from the valves. Thus the valves represented a calcium reserve for *Anodonta cygnea*. It is of interest to note that there is an account by DUGAL (1939) of the well-regulated use of the calcareous shell of *Venus mercenaria* for buffering the products of glycolysis during periods of anaerobic metabolism. Phenomena of these kinds may influence the use of freshwater lamellibranchs for monitoring purposes, perhaps particularly in waters oligotrophic with respect to calcium and in climates with long winters.

The phenomenon of valve corrosion has been given little or no attention in the literature on the use of lamellibranchs for monitoring purposes. It is an old established fact that valves of freshwater lamellibranchs are liable to corrosion. Several external agents are responsible for this action. Corrosion of shells may be brought about by mechanical, chemical or biotic factors, or a combination of these. The ecological aspect of valve corrosion is described by WESENBERG-LUND (1937, p. 788). Of the more than a hundred specimens of *Anodonta piscinalis* examined for the present investigation only very few showed no signs of valve corrosion. In environments such as those described in this paper this factor may be a complication for the use of *Anodonta piscinalis* as indicator of long term contamination with low-level radioactive wastes.

The practical use of freshwater lamellibranchs in monitoring programmes of contaminated inland waters appears promising. But there are complicating factors whose nature is only poorly understood at present. Physiological studies on the mineral metabolism of the lamellibranchs as related to development and varying environmental conditions should be encouraged.

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SUMMARY

The present investigation has been concerned with the accumulation of radionuclides by the freshwater lamellibranch *Anodonta piscinalis* NILSSON. Specimens of the animal were exposed for 125 days to approximately constant concentrations of the radionuclides P^{32}, Sr^{89}, Cs^{137}, Ce^{144} and Zr^{95}/Nb^{95}; in addition Ru^{103} was included among the radionuclides for the last 55 days of the experiment. The investigation was conducted in a model recipient with environmental factors similar to those existing in the animals' biotope in nature.

The accumulation of the radionuclides was measured in the body and valves of the lamellibranch. The results indicate that an approximately constant level of radioactivity was reached after one month's exposure in these two parts of the animal. Subsequently the level of radioactivity showed a fluctuating pattern which was ascribed to seasonal and physiological changes. Accumulation of the radionuclides in the test animals varied according to the following sequence, arranged in order of decreasing affinity: $P^{32} > Sr^{89} > Ce^{144} > Ru^{103} > Cs^{137} > Zr^{95}\ Nb^{95}$. Factors which may complicate the practical use of freshwater lamellibranchs in the monitoring of contaminated recipients are pointed out and discussed.

ZUSAMMENFASSUNG

Die vorliegende Untersuchung betrifft die Anhäufung von Radioisotopen bei der Lamellibranchiate *Anodonta piscinalis* NILSSON.

Exemplare dieses Tieres wurden beobachtet während 125 Tagen bei annähernd konstanter Konzentration der Isotopen P^{32}, Sr^{89}, Cs^{137}, Ce^{144} und $Zr^{95}\ Nb^{95}$, während 55 Tagen des Experimentes wurde Ru^{103} miteinbezogen.

Das Experiment wurde in einem Flussmodell durchgeführt bei gleichen Umgebungsfaktoren wie sie das Tier in seinem natürlichen Biotop findet.

Die Anhäufung der radioaktiven Isotope wurde im Körper und in der Schale der Lamellibranchiate gemessen.

Das Resultat erwies, dass innerhalb eines Monats das Tier eine gewisse Radioaktivität erreichte in den zwei geprüften Teilen. Das Radioaktivitätsniveau wies eine wellenartige Schwankung auf, welche dem Jahreszeitlichen und physiologischen Wechsel zuzuschreiben ist.

Die Radioisotope wurden im Tier in folgender Reihenfolge angehäuft: $P^{32} > Sr^{89} > Ce^{144} > Ru^{103} > Cs^{137} > Zr^{95}\ Nb^{95}$.

Faktoren komplizierter Natur sind für den praktischen Gebrauch der Süsswasserlamellibranchiata in der Radioaktivitätsüberwachung verunreinigter Gewässer begrenzend, und werden hier diskutiert.

REFERENCES

BERG, A., MERLINI, M., RAVERA, O. & TONOLLI, V.: International Atomic Energy Agency, Research Contract No. 59, Final Report. October 1961.

BEVELANDER, GERRIT: Calcification in molluscs. III. Intake and deposition of Ca^{45} and P^{32} in relation to shell formation. *Biol. Bull.* 102, February-June 1952.

DUGAL, LOUIS-PAUL: The use of calcareous shell to buffer the product of anaerobic glycolysis in *Venus mercenaria. J. Cell. comp. Physiol.*, 13, February, April, June 1939.

EHRMANN, P.: Weichtiere, Mollusca. *Tierwelt Mitteleuropas*, 2. 1, 1933.

FRETTER, VERA: Experiments with radioactive strontium (90 Sr) on certain molluscs and polychaetes. *J. mar. Biol. Ass. U.K.*, 32, 1953.

JODREY, LOUISE H.: Studies on shell formation. III. Measurement of calcium deposition in shell and calcium turnover in mantle tissue using the mantle-shell preparation and Ca^{45}. *Biol. Bull.* ,104, February-June 1953.

JODREY, LOUISE H., & WILBUR, KARL M.: Studies on shell formation. IV.

The respiratory metabolism of the oyster mantle. *Biol. Bull.*, 108, February-June 1955.

KADO, YÔICHI: Studies on Shell Formation in Molluscs. *J. Sci. Hiroshima Univ.*, Series B. Div. 1, 19, November 1960.

MANDAHL – BARTH. G.: Bløddyr III. Ferskvandsbløddyr. *Danmarks Fauna.* 54, 1949.

MORTON, J. E.: *Molluscs.* London 1958.

NELSON, D. J.: Clams as indicators of strontium - 90. *Science*, 137, No. 3523, 6. July 1962.

PICKEN, L. E. R.: The mechanism of urine formation in invertebrates. II. The excretory mechanism in certain *Mollusca. J. exp. Biol.*, 14, 1937.

POLIKARPOV, G. G.: Accumulation of the radio-isotope of cerium by fresh-water molluscs. *DEG Information Series* 102 (W). United Kingdom Atomic Energy Authority, 1960.

PROSSER, LADD C. & BROWN, FRANK, A.: *Comparative Animal Physiology.* London 1961.

RAO, K. PAMPAPATHI & GOLDBERG, EDWARD, D.: Utilization of dissolved calcium by a pelecypod. *J. Cell. comp. Physiol.*, 43, February, April, June, and Supplement No. 1 to Vol. 43, May 1954.

RAVERA, OSCAR & VIDO, LUDOVICO: Misura del Mn – 54 in popolazioni di *Unio pictorum* L. (Molluschi, Lamellibranchi) del Lago Maggiore. *Mem. Ist. Ital. Idrobiol.* 13, 1961.

TIMOFEEV-RESOVSKII, N.V. & TIMOFEEVA-RESOVSKAIA, E. A.: Coefficients d'accumulation des isotopes radioactifs de 16 éléments différents par les organismes d'eau douce et influence du complexon EDTA sur certains d'entre eux. Commissariat á L'Energie Atomique, Traduction No. R. 1243, Date: 6.4 1961.

VINOGRADOV, A. P.: The elementary chemical composition of marine organisms. *Mem. Sears Found. Mar. Res.*, Number II, New Haven, 1953.

WAELE, A. DE: Le sang d'*Anodonta cygnea* et la formation de la coquille. Académie Royale de Belgique. Classe des Sciences. 2. Série 10, 1929.

WESENBERG-LUND, C.: Ferskvandsfaunaen biologisk belyst. Invertebrata. Andet bind, København 1937.

WORLD HEALTH ORGANIZATION: Methods of radiochemical analysis. Report of a joint WHO/FAO expert committee. WHO, Technical Series No. 173, Geneva 1959.

ØKLAND, JAN: Notes on population density, age distribution, growth, and habitat of *Anodonta piscinalis* NILSS. (Moll., Lamellibr.) in a eutrophic Norwegian lake. *Nytt Mag. Zool.* ,11, 1963.