COMPARATIVE BIOCHEMICAL STUDIES ON AQUATIC ANIMALS. I. CALCIUM TURNOVER OF THE FRESHWATER FISH AND SHELLFISH

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I. CALCIUM TURNOVER OF THE FRESHWATER FISH AND SHELLFISH.*

By

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Introduction

Up to the present, there are many investigations concerning the calcium metabolism of terrestorial animals. With regard to aquatic animals, Wilbur and Jodrey (1) and Jodrey (2) have reported on the shell formation using the oyster mantle shell preparation and Ca^{45}. Tomiyama et al (3) and Horiguchi et al (4) have studied the same subject taking into consideration the freshwater fishes (goldfish and prussian carp) and the freshwater shellfish (Hyriopsis schlegelii v. MARTENS). For elucidating the mechanism of the calcification in vivo, correlation between the mineral constituents (Ca, P, N) and the relating enzymic systems must be clarified.

In the present report, we have examined in the first place the accumulation, excretion and decontamination of Ca^{45}, employing the goldfish and the pond mussel as the experimental animals. The authors are indebted to Prof. Hatai for his kind identification of the shellfish. They also wish to thank Prof. Tsuchiya for his criticism concerning this study. The expense of the study was partly aided by a grant from the Ministry of Education, for which they express their hearty thanks.

Experimental

(1) **Materials.** The common goldfish (*Carassius auratus* L.) and the pond mussel (*Anodonta lauta* MARTENS).

(2) **Methods.** **The simultaneous quantitative determination of Ca^{40} and Ca^{45}.**

Procedure: After the various time intervals of culture in the radioactive pond water, the animals are sacrificed, dissected and separated into each tissue

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groups. The pooled samples of each tissue groups are placed in the crucibles, weighed and are subjected to wet ashing (sometimes with fumic nitric acid or hydrogen peroxide). A part of each tissue groups is also used for the determination of the moisture contents. Ash of a tissue is dissolved into 1N HCl solution and heated to expel the carbon dioxide. A few drops of methyl orange indicator is added to the solution. After neutralization with ammonium hydroxide, 1 ml of dil. HCl (3N) is added to make it slightly acid. When it is again heated to boiling, hot saturated ammonium oxalate solution is added. After the addition of ammonium hydroxide to make it alkaline, the solution is boiled for ten minutes and left to stand for about half an hour. Calcium oxalate precipitates formed are filtered and collected in homogeneous geometry on the filter paper put on the fritted disk of the filtrating apparatus by suction. Then they are washed with hot dil. ammonium hydroxide solution (1:50) and washed with small amounts of alcohol to facilitate the drying faster. The precipitates on the filter paper are transferred to a counting dish (made of stainless steel) and dried under the infrared lamp. Radioactivity of the precipitates are counted for a given time intervals with the Geiger Mueller counter. Thereafter, the precipitates are dissolved into 1N sulfuric acid and warmed to 70°C in the water bath and immediately titrated with N/10 $KMnO_4$ solution. (determination of the total calcium, $Ca^{40}+Ca^{45}$).

(3) Preliminary experiments to establish the cultural conditions.

To establish the experimental conditions suitable for the culture, the three important factors, i. e. the water temperature, the quality of the cultural water and the diet of the experimental animals, were analysed under the designed conditions as indicated in Table 1. Summarizing the results obtained, the better growth was observed under the following conditions; a lower temperature

Table 1. Preliminary experiments to establish the cultural conditions.

Fraction	Water temp.	Cultural media	Diet	Interval of culture	Average growth after culture
(1) (A)	High temperature (30±1°C)	Pond water	Wheat gluten(200mg) Pupa powder(300mg)	21 days	5.56 g
(B)	Low temperature (16±1°C)	,,	,,	,,	6.64 g
(2) (A)	21±4°C*	Pond water	The same as above	22 days	7.30 g
(B)	,,	Tap water	,,	,,	6.20 g
(3) (A)	21±4°C*	Pond water	Pupa only (500mg)	27 days	6.30 g
(B)	,,	,,	Wheat gluten only (500mg)	,,	6.00 g

Remarks; * The temperature was according to that of the room.
Three liters of the media were exchanged once every day. The Ca content of the pond water was 11.4–13.1 mg Ca per liter and those of the diet were 0.83 mg Ca per g of dry matter (wheat gluten) and 7.0–7.9 mg Ca per g. of dry matter (pupa powder).

(16±1°C) was better than a higher temperature (30±1°C), the pond water surpassed the tap water, and the pupa powder of the silkworm was more favorable than the light cake made of wheat gluten (Fu). Considering the results of Table 1 and the conveniences for the experiment, we decided to carry out the animal culture in the tracer experimens under the following conditions; pond water, temperature (21±4°C), and a mixed diet of the pupa powder and the light cake made of wheat gluten (300 mg : 200 mg per one vat). We have also examined the lethal dose of $CaCl_2$ to the experimental animals, since the radioactive isotope (Ca^{45}) available is in the form of $CaCl_2$ in diluted acid. The results of the experiments are given in Table 2.

Table 2. The lethal dose of the experimental animals to $CaCl_2$.

Animals	Concentration of $CaCl_2$	Time from the administration to death	Remarks
Goldfish	1N	17 min.	Whitening of the whole body surface after 3 min. Blood diffuse out from the gill. Lose balance of the body.
	0.5N	25 min.	
	0.25N	1h 15 min.	
	0.125N	41h 45 min.	Water temperature 23±1.5°C
	0.1N	Tolerate within the experimental period.	The goldfish of 3–4 g body weight was used.
Pond mussel	0.5N	2h	Water temperature 25°C.
	0.25N	5h	
	0.1N	24h	
	0.05N	72h	
	0.025N	Tolerate within the experimental period.	
	0.0125N	The same as above.	

The lethal dose of the experimental animals to $CaCl_2$ ranges from 0.025N (1.84 mg Ca/ml) to 0.1N (7.35 mg Ca/ml). Since the original solutions of Ca^{45} (ranging from 53 mg Ca/ml to carrier free) were diluted more than 1000 fold in the experiment, such a low concentration of $CaCl_2$ does not affect the results of the experiments.

(4) Counting and calculation of the radioactivity.

Counting of the radioactivity of the samples in the counting dish was made with the Geiger Mueller counter GM131 (mica window thickness 2.5–3.0 mg/cm^2) and GM132 (mica window thickness 1.8 mg/cm^2), especially with the latter unless otherwise stated. The distance between the sample and the window was 16 mm. As the statistical error of the counting of the radioactivity is proportional to the root of the total counts, the error is calculated as about one per cent in the case of 10000 cpm total counts, and as about three per cent in the case of 1000 cpm total counts. We have counted, therefore, more than 10000 cpm in the case of the high leveled sample and usually at least more than 1000 cpm

to bring the error within three per cent. However, in the case of the very low leveled one whose activity was as low as the natural counts, the counting for more prolonged time (30 min. or more) was carried out to minimize the error as far as possible. For the relative comparison of the counts of each tissue groups, the effect of self absorption on the counts should be considered. For that purposes, we have carried out as follows; The total Ca (in mg) is divided by the surface area (geometry area 2.8 cm^2) and the quotient indicates the sample thickness. Then the correction factor (f) corresponded to the sample thickness is obtained graphically from the calibration curve (Figure 1)

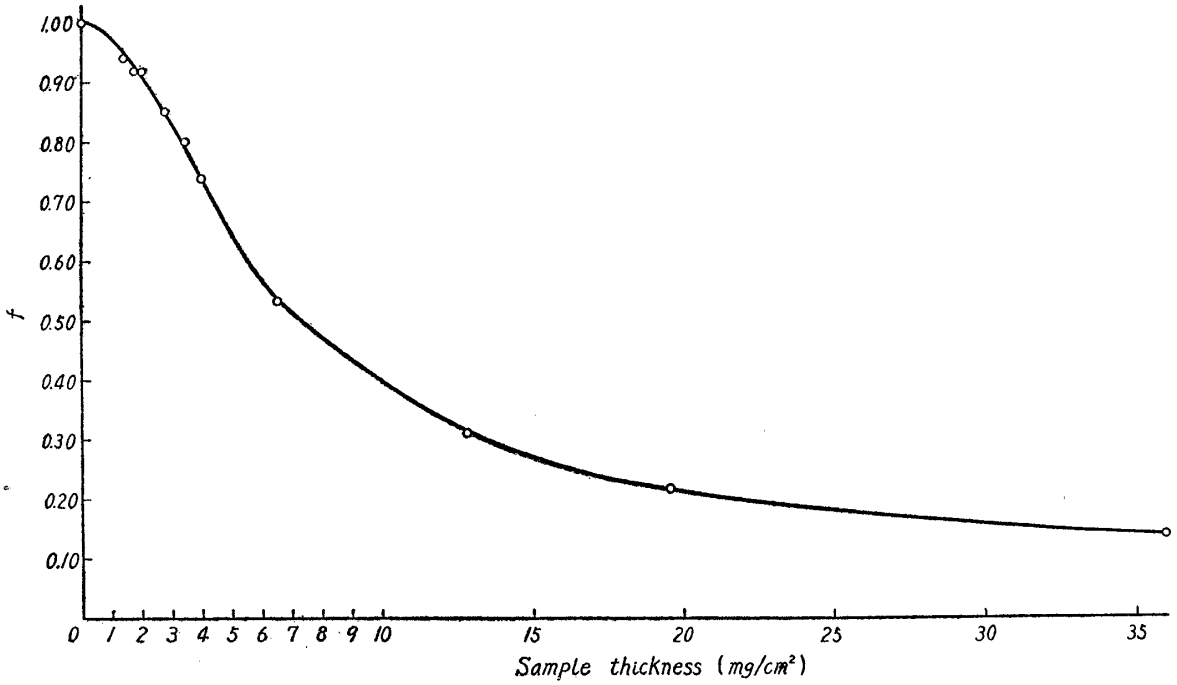

Fig. 1. Calibration curve to correct the self absorption (Ca^{45}).

made previously under our conditions. Then we have calculated the specific activity, S. A., by the following formula;

$$\text{S. A.} = C/f \times 1/Ca \text{ mg in the sample}, \quad (1)$$

where C is the measured cpm, f is the correction factor for a sample thickness. We have also expressed the radioactivity as $C_{Calc.}$. We have defined $C_{Calc.}$ as "the counts per minute of calcium precipitates contained in 1 g dry matter tissue at 0 mg/cm^2 of the sample thickness". (extraporated to 0 mg/cm^2 sample thickness) It can be formulated as follows;

$$C_{Calc.} = C/f \times I/W, \quad (2)$$

where W is the dry matter weight of the sample tissue analyzed.

(5) **The distribution of the total calcium (Ca^{40}) in the tissues of the experimental animals.**

As a preliminary step before the tracer experiment, the distribution of the

total calcium in the tissues of the animals was studied as shown in Table 3.

Table 3. The distribution of the total calcium in the tissues of the experimental animals. (expressed as Ca mg/1g dry tissue)

	Gill	Operculum	Scale	Fin	Bone	Muscle	Total viscera
Goldfish	43.95 *74.92%	104.69 71.38%	67.65 65.43%	148.90 74.75%	130.29 64.21%	5.29 78.96%	4.07 70.69%
Prussian carp	54.42 *74.54%	158.55 57.42%	140.92 33.56%	211.77 55.18%	160.33 53.36%	4.79 79.36%	7.34 86.81%
Pond mussel	Gill	Mantle edge	Mantle interior	Digestive diverticula	Adductor muscle	Foot	Gonad (Viscera)
	117.40 *86.76%	19.78 86.66%	24.82 88.75%	12.47 84.90%	4.01 86.44%	2.69 85.30%	9.38 81.46%

Remarks ; * These figures indicates the moisture contents of the tissues.

(6) Accumulation of Ca^{45} in animals.

(A) Goldfish

For the accumulation of Ca^{45} in the goldfish, five fishes per one vat were cultured in 2 liters of the pond water with a Ca^{45} activity of 2340 cpm/ml. The basal diet (pupa-wheat gluten mixture) was given once daily. After the various time intervals, the animals were killed, dissected and separated into each tissue

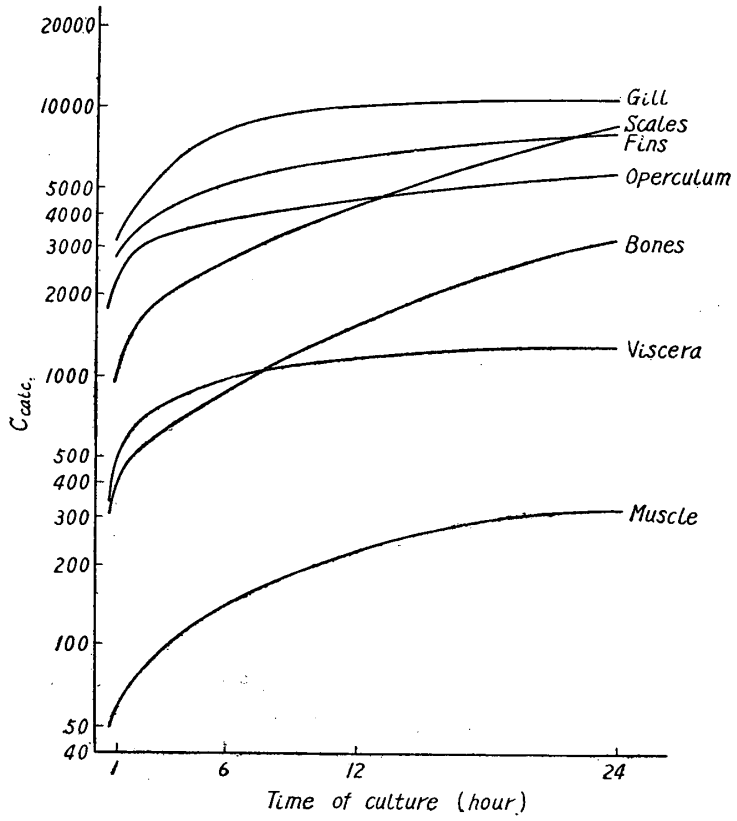

Fig. 2. The accumulation curves of Ca^{45} in the tissues of the goldfish.

groups. From the pooled sample of each tissue groups, the sample was taken and analysed as before described. The results are shown in Table4 and Figure 2.

Table 4. Accumulation of Ca^{45} in the goldfish.

Tissues	Time of culture	$C_{Calc.}$ △	S. A. *
Gill	1h.	3429±73	42
	3	2919±94	70
	6	8283±195	79
	24	10948±129	206
Operculum	1h.	3260±282	3
	3	3234±220	8
	6	1190±70	9
	24	5872±224	20
Bone	1h.	394±93	1
	3	616±70	2
	6	656±49	2
	24	3288±116	11
Fin	1h.	3154±151	6
	3	4656±203	16
	6	3544±145	13
	24	8107±181	41
Total viscera	1h.	675±15	399
	3	701±16	952
	6	2333±47	816
	24	1258±21	1243
Muscle	1h.	109±12	9
	3	72±11	15
	6	139±9	29
	24	333±21	48
Scales	1h.	1372±61	8
	3	1464±63	10
	6	2781±86	19
	24	8284±129	59

Remarks ; The experiment was carried out on the 24th-26th August 1954.
GM counter 131. water temp. 25°C. Body weight of the goldfish used, 6–7g.
△ Calculated from the formula (2). * Calculated from the formula (1).

Considering from the $C_{Calc.}$ in Table 4, the accumulation of Ca^{45} in the tissues of the goldfish were active in the following order (decreasing order) ; gill, scales or fins, operculum, bones, viscera, muscle. The results are illustrated graphically in Figure 2. However in regard to the specific activity, it does not always follow the above order. In the tissues containing a small quantity of total calcium such as viscera and muscle (4–5 mg Ca/g. dry tissue), the incorporation of the small quantity of Ca^{45} resulted in the rapid increase of the specific activity, while in the calcified tissues such as bones and fins (130–140 mg Ca/g. dry tissue) the incorporation of Ca^{45}, even if the net uptake is much higher, did not result in the rapid increase of the specific activity but in the slow increase. Therefore, the illustration of the results expressed in $C_{Calc.}$ is more

suitable for the quantitative comparison than in the specific activity. It is noteworthy that the viscera showed extraordinary higher specific activity.

(B) Pond mussel

For the accumulation of Ca^{45} in the pond mussel, five shellfishes per one vat were cultured in 2 liters of the pond water with an activity of 2617 cpm/ml. No diet was given particularly. After intervals, the animals were killed and treated in the same way as the goldfish. The results are shown in Table 5 and Figure 3.

Table 5. The accumulation of Ca^{45} in pond mussel.

Tissues	Time of culture	$C_{Calc.}$	S. A.
Mantle edge	1h.	5240±120	144
	4	10408±129	528
	8	8762±182	298
	24	13794±304	448
Mantle interior	1h.	2605±62	123
	4	10442±152	171
	8	13680±245	226
	24	30986±368	695
Gill	1h.	11695±208	96
	4	18311±287	134
	8	13024±242	101
	24	38532±568	210
Foot	1h.	446±17	60
	4	4843±135	213
	8	2401±55	263
	24	4420±66	906
Gonad (Viscera)	1h.	1686±32	150
	4	4536±72	249
	8	4802±83	162
	24	10322±109	167
Digestive diverticula	1h.	1915±39	81
	4	8554±143	109
	8	14602±235	158
	24	33264±221	807
Adductor muscle	1h.	1548±33	161
	4	3146±82	251
	8	8553±154	1192
	24	7180±113	717

Remarks ; The experiment was carried out on the 7th-14th September 1954.
Water temp. 25°C. Medium sized shellfishes (70–80 g with shell) were used.

The accumulation of Ca^{45} in pond mussel was reexamined in winter (February). In this case, five shellfishes per one vat were cultured in 2 liters of the pond water with an activity 400 cpm/ml. The temperature during the culture was adjusted to 16–17°C. After intervals, the animals were treated as before. The results are shown in Table 6.

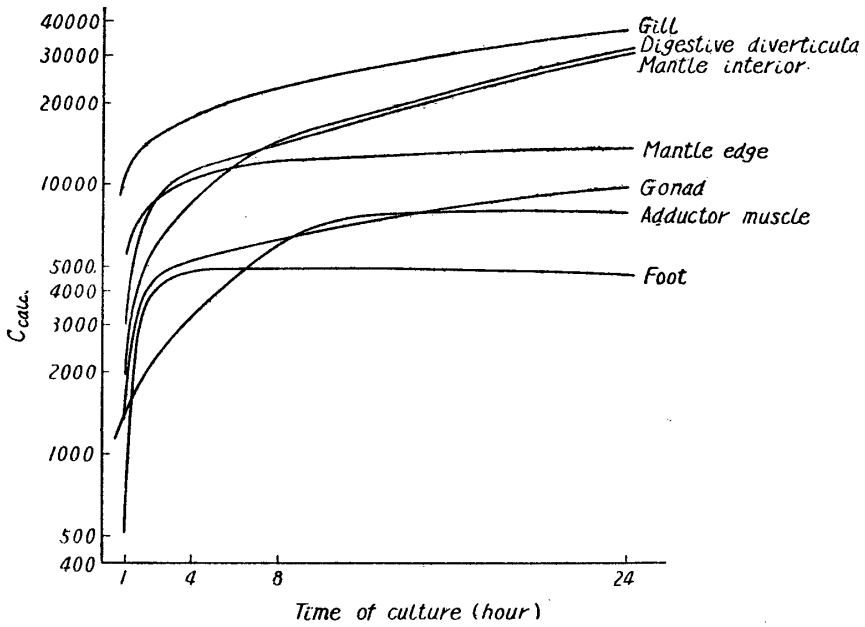

Fig. 3. The accumulation curves of Ca^{45} in the tissues of the pond mussel.

Table 6. The accumulation of Ca^{45} in Pond mussel in winter.

Tissues	Time of culture	S. A.
Mantle edge	4h.	194
	8	287
	24	298
Mantle interior	4h.	119
	8	208
	24	288
Gill	4	34
	8	66
	24	143
Foot	4	158
	8	242
	24	297
Gonad	4	86
	8	237
(Viscera)	24	183
Digestive diverticula	4	60
	8	192
	24	186
Adductor muscle	4h.	148
	8	640
	24	604

Remarks: The experiment was carried out on the 19th Jan-8th Feb 1955.
Water temperature 16–17°C (adjusted)
Average body weight of the shellfish is 102g.

The accumulation of Ca^{45} in the tissues of the pond mussel were active in the following order when expressed in $C_{Calc.}$; gill, mantle interior or digestive diverticula, mantle edge, gonad, adductor muscle, foot. However, when expressed in the specific activity, the tissues containing a small quantity of total calcium such as foot and adductor muscle show higher specific activity than the other tissues with much quantity of total calcium from the same reason as before described.

Ca^{45} renewal in goldfish.

If we assume that the greater part of the Ca^{45} uptake is completed and the radioactive equilibrium between the animals and the medium is established after a given period, a comparison of the specific activities of the tissues and the medium allow one to calculate the amount of Ca^{45} which has been renewed in the tissues during this interval. The renewed Ca^{45} during the intervals is expressed approximately by the following formula.

Renewed Ca^{45} in the tissues

$$= S.A._{tissue}/S.A._{medium} \times 100 \times \text{total Ca (mg/1g.wet tissue)}$$
$$= \%\ \text{of renewing fraction} \times \text{total Ca} \qquad (3)$$

The renewed Ca^{45} in the tissues of the goldfish can be calculated from the formula (3) on the assumption that the radioactive equilibrium is attained after the culture for 24 hours. The results are shown in Table 7.

Table 7. Renewal of Ca^{45} in goldfish.

Tissues	S. A.*	% of renewing◎ fractions	Total calcium in 1 g wet tissues	Ca^{45} weight to be renewed in 1g wet tissues until the equilibrium.
Gill	206	0.58	10.74 mg	0.062 mg
Operculum	20	0.06	35.73	0.022
Fins	41	0.11	37.79	0.042
Bones	11	0.031	48.09	0.015
Viscera	1243	3.50	0.35	0.012
Muscle	48	0.13	1.10	0.001
Scales	59	0.16	25.39	0.041

Remarks ; * The specific activities of the tissues after the culture for 24 hrs.
◎ Per cent of renewing fractions, obtained by dividing the S. A. of the tissues by the S. A. of the radioactive pond water (35454).

Ca^{45} renewal in pond mussel.

The renewal of Ca^{45} in the pond mussel is calculated in the same manner as in the case of the goldfish. The results are given in Table 8.

The accumulation of Ca^{45} in the shell of the pond mussel.

The accumulation of Ca^{45} in the shell of the pond mussel was examined as follows; After the intervals of culture the shell was attached under a metal plate with cellophane tape. The metal plate has a circular hole (diameter 10mm) in the center. The emmitted β radiations from the Ca^{45} deposited in the inner

Table 8. Renewal of C^{45} in pond mussel.

Tissues	S. A.	% of renewing fractions	Total calcium in 1 g wet tissues	Ca^{45} weight to be renewed in 1 g. wet tissues until the equilibrium
Mantle edge	448	1.12	3.33 mg	0.037 mg *
Manlte interior	695	1.75	3.81	0.066
Gill	210	0.53	18.80	0.0996
Foot	906	2.28	0.34	0.008
Gonad (Viscera)	167	0.42	3.02	0.013
Digestive diverticula	807	2.03	3.26	0.066
Adductor muscle	717	1.81	0.66	0.012

Remarks; * The calculation was carried out on the assumption that the radioactive equilibrium is established after the culture for 24 hours. The specific activity of the radioactive pond water was 39651.

shell surface pass through the center hole of the plate and the radioactivity was measured with the counter. The results obtained are shown in Table 9A. However, the counting of the radioactivity was insufficient owing to the scattering of the β radiations by the uneven curvature of the inner shell surface. Then the shell with Ca^{45} was minced in the mortar, weighed and then ashed. The subsequent procedure was carried out in the same way as in the case of the soft tissues. The results are given in Table 9B.

Table 9A. The deposition of Ca^{45} in the inner shell surface.

Time of depositin	1 h	4 h	8 h	24 h
Intact shell	7 cpm	15	10	25

Table 9B.

Days	C_{Calc}	S. A.	% of renewing fractions	Total Ca mg per 1 g wet shells	Renewed Ca^{45} in 1 g wet shells
1	326±38	1.11	0.03	383.06 mg	0.115 mg
2	588±39	1.56	0·04	,,	0.153
3	3264±325	1.52	0.04	,,	0.153

Remarks; The activity was expressed in cpm per 2.5cm^2 inner shell surface.
The specific activity of culture media was 3800.

(7) The disappearance of Ca^{45} from the animals.

(A) Goldfish

Five fishes per one vat were cultured for three days in 3 liters of the pond water with a Ca^{45} activity of 1012 cpm/ml in the same manner as in the accumulation. The basal diet was given daily. After 3 days' accumulation, test fishes were transferred to the non-radioactive isotonic pond water once daily. After intervals, the animals were killed and the radioactivity retained in the animal tissues was measured as before. The results are indicated in Table 10 and Figure 4.

Table 10. The disappearance of Ca^{45} from the goldfish.

Tissue	Time of culture	CCalc	S. A.
Gill	0 h *(immediately after the accumulation.)	31746±263	746
	6	22830±220	588
	24	35504±352	433
	72	23647±229	410
Operculum	0	32591±329	169
	6	15041±164	81
	24	6020±78	78
	72	13024±135	82
Fins	0	28356±284	228
	6	19738±196	154
	24	18168±179	221
	72	12555±119	118
Bones	0	21723±216	173
	6	12610±100	80
	24	14504±145	87
	72	13741±133	76
Viscera	0 h	1293±13	708
	6	1154±12	648
	24	710±12	281
	72	237± 7	142
Muscle	0	1539±15	424
	6	642±11	162
	24	1857±33	178
	72	1911±19	198
Scales	0	44554±445	489
	6	29943±275	341
	24	28556±177	261
	72	33987±326	268

Remarks ; The experiment was carried out on the 5th–7th October 1954. Water temp. 15.5°C. Average body weight of the goldfish is 9.0 g.
* 0h means the sample of the goldfish immediately after 3 days' accumulation which was used as a control in the disappearance of Ca^{45}.

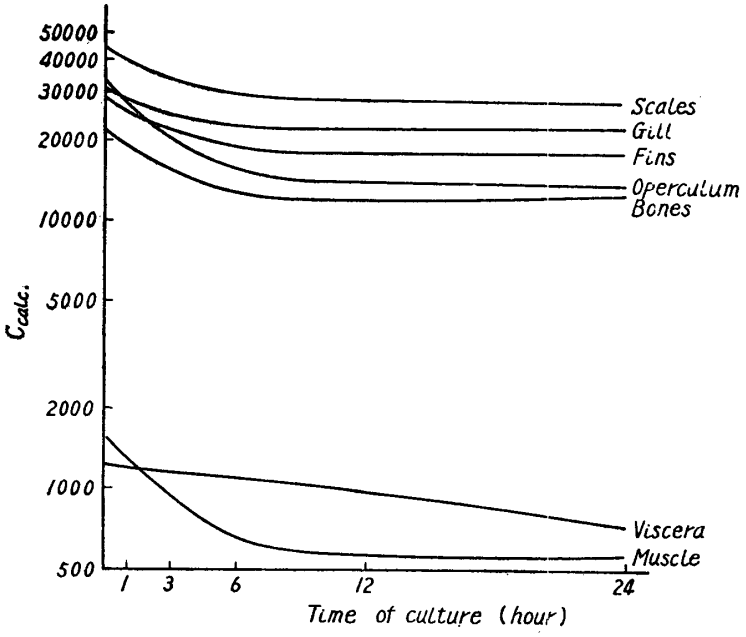

Fig. 4. The disappearance curves of Ca^{45} in the tissues of the goldfish.

(B) Pond mussel

Five shellfishes per one vat were cultured in 3 liters of the pond water with a Ca^{45} activity of 644 cpm/ml (from the 1st day to the 3rd day of culture) and 422 cpm/ml (the 4 th day) for the accumulation of the isotope. After 4 days' accumulation, the test animals were transferred once daily to the non-radioactive isotonic pond water. After intervals, the animals were analyzed in the same way as the goldfish. The results are shown in Table 11 and Figure 5.

Table 11. The disappearance of Ca^{45} from the pond mussel.

Tissues	Time of culture	CCalc.	S. A.
Mantle edge	0 h	25027±218	1482
	24	16450±163	980
	72	23100±228	673
Mantle interior	0	45612±441	1383
	24	51831±509	1031
	72	35218±308	575
Gill	0	70022±627	600
	24	87692±399	574
	72	43350±421	319
Foot	0	1600±32	308
	24	2815±30	735
	72	1140±24	432
Gonad (Viscera)	0	37679±349	1526
	24	25421±220	646
	72	20562±179	399
Digestive diverticula	0	42945±379	1547
	24	26467±261	747
	72	23513±231	513
Adductor muscle	0	26290±262	1269
	24	6700± 70	763
	72	4506± 45	574

Remarks; The experiment was carried out on the 25th–28th October 1954. Water temp. 13–15°C. Average body weight of the shellfish is 22.7g.

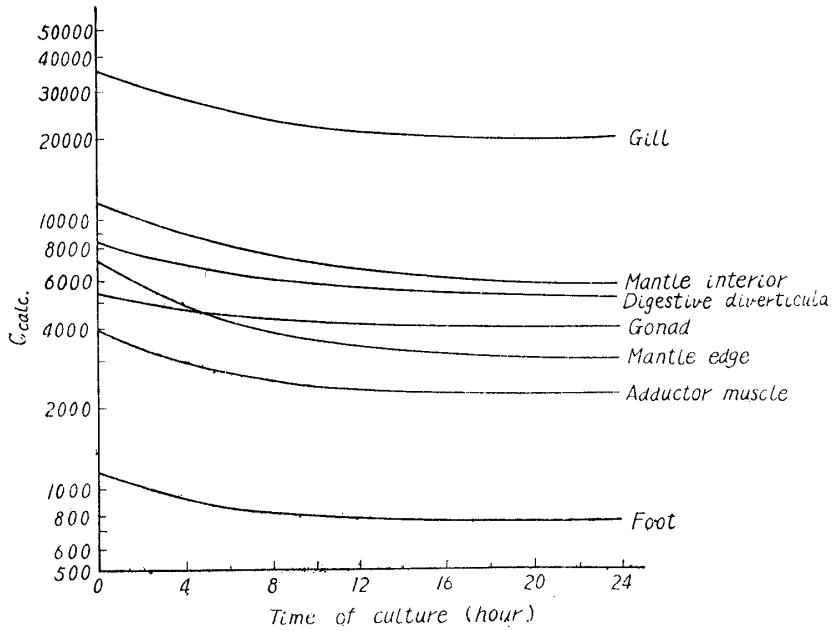

Fig. 5. The disappearance curves of Ca^{45} in the tissues of the pond mussel.

The disappearance of Ca^{45} from the pond mussel was reexamined in winter. In this case, five shellfishes per one vat were cultured in 2.25 liters of the pond water with a Ca^{45} activity of 453 cpm/ml. The temperature was adjusted at 16° C. After the accumulation of Ca^{45}, the treatment of the sample was the same as before. The results are shown in Table 12.

Table 12. The disappearance of Ca^{45} from the pond mussel in winter.

Tissues	Time of culture	S. A.	C Calc. *
Mantle edge	0h (control)	565	7140
	24	333	3000
	48	353	4829
	96	142	3528
Mantle interior	0	479	10160
	24	323	5800
	48	278	6084
	96	211	4450
Gill	0	323	35211
	24	178	20000
	48	228	22749
	96	123	18669
Digestive diverticula	0	508	8322
	24	339	5017
	48	393	
	96	211	5775
Gonad (Viscera)	0	593	5322
	24	357	4068
	48	408	4947
	96	209	2677
Foot	0h	359	1155
	24	185	773
	48	141	646
	96	220	659
Adductor muscle	0	410	3977
	24	300	2200
	48	321	2234
	96	129	1633

Remarks; The experiment was carried out on the 24th February-1st March 1955. Water temperature 16°C. Average weight of the shellfish (with shell) is 61.5g.
The calculation of error was omitted.

By the semi-logarithmic plot of the rate of disappearance of Ca^{45} from the tissues, i. e., by plotting the specific activity on the logarithmic scale and by plotting the time of culture on the arithmetic scale, we can obtain the biological half time (τ) graphically. From the τ value, the turnover time is calculated. Further from the turnover time, the turnover rate is calculated. The results are given in Table 13 (goldfish) and in Table 14 (pond mussel).

Table 13. Turnover of Ca^{45} in the goldfish.

Tissues	Half time (τ)	Turnover time (τ/0.693)	Ca^{45} to be renewed in 1g wet tissues until the equilibrium	Turnover rate(mg Ca^{45}/hour/1g wet tissues)
Gill	18h (1h)	25.9h (1.4h)	0.062mg	0.0024mg (0.044)
Operculum	6 (3)	8.6 (4.3)	0.022	0.0025 (0.0051)
Fins	11 (3.5)	15.8 (5)	0.042	0.0026 (0.0084)
Bones	5.5 (2.0)	7.9 (2.8)	0.015	0.0018 (0.0053)
Viscera	18	25.9	0.012	0.0004
Muscle	4 (3.5)	5.7 (5.0)	0.001	0.00017 (0.0002)
Scales	11.5 (2.5)	16.5 (3.6)	0.041	0.0024 (0.0114)

Remarks: Figures in the parentheses are obtained on the assumption that the initial rapid disappearance rate (from 0h to 3h) being held during the experiment, although the data of 3 hours' disappearance are omitted.

Table 14. Turnover of Ca^{45} in the pond mussel

Tissues	Half time (τ)	Turnover time (τ/0.693)	Ca^{45} to be ronewed in 1g wet tissues until the equilibrium	Turnover rate(mg Ca^{45}/hour/1g wet tissues)
Mantle edge	32h	46.1h	0.037mg	0.0008mg
Mantle interior	42	60.6	0.066	0.0011
Gill	28	40.4	0.0996	0.0025
Foot	25	36.0	0.008	0.0002
Gonad (Viscera)	33	47.6	0.013	0.0003
Digestive diverticula	40	57.7	0.066	0.0011
Adductor muscle	50	72.1	0.012	0.0002

(8) The decontamination of Ca^{45} from the animals.

The use of ethylenediamine tetraacetic acid (EDTA di-or tetrasodium salts) is already recommended for the decontamination of Ca^{45} from the animals, and Cohn et al (5) reported that the pre-treatment with EDTA is more efficient for the decontamination in the case of the rat than the post-treatment of the animals with EDTA. However, the pre-treatment of the aquatic animals with such chemicals is practically impossible. The decontamination of Ca^{45} from the aquatic animals, therefore, means the post-treatment of the contaminated animals with such chemicals. Preliminary experiments on the lethal dose of Na_4 EDTA to the goldfish showed that the critical dose is about 200mg per

liter of the culture medium.

(A) Prussian carp (Decontamination from the living animal)

Prussian carp (body weight about 120g) was placed in 4 liters of the pond water with a Ca^{45} activity of 39 cpm/ml for 2 days (one carp per one vat). After the accumulation of Ca^{45}, the carp was replaced into the non-radioactive isotonic pond water added with 200mg Na_4 EDTA. This medium was exchanged once daily for the elution of the Ca-EDTA complex. After the elution for 4 days and 6 days, the animals were analyzed as before described. The results are shown in Table 15.

Table 15. Decontamination of Ca^{45} from the prussian carp by the EDTA treatment.

Treatment	Tissues	C Calc	S. A.
No treatment (control, after 2 days' accumulation)	Gill	1894±32	33
	Operculum	272±27	2
	Scales	1246±35	10
	Fins	687±34	4
	Bones	222±20	2
	Muscle	134± 6	69
EDTA treatment for 4 days	Gill	917± 3	13
	Operculum	223±27	1
	Scales	376±22	3
	Fins	190±28	1
	Bones	322±57	1>
	Muscle	35± 4	6
EDTA treatment for 6 days	Gill	442± 2	7
	Operculum	551±47	2
	Scales	385±19	4
	Fins	383±44	1
	Bones	116±20	1>
	Muscle	16± 3	7

Remarks ; The experiment was carried out on the 18th November1954. Water temperature 10–12°C.

(B) Pond mussel (Postmortem decontamination from the animals)

For the accumulation of Ca^{45}, five shellfishes were cultured for a day in the pond water with an activity of 623 cpm/ml. After the accumulation, they were killed, dissected and grouped into each tissues separately. The pooled sample of each tissues (gill, foot, digestive diverticula and gonad) were immersed into the several portions of 0.5 per cent Na_2 EDTA solution (total volume 300ml) for 4 hours and shaken frequently. After the elution of the Ca-EDTA complex, the radioactivity retained in the tissues were measured as before described. The radioactivity of the control animals, washed once with

the non-radioactive pond water, were measured without the EDTA treatment. The results are shown in Table 16.

Table 16. Postmortem decontamination of Ca^{45} from the poned mussel.

Treatment	Tissues	C Calc	S. A.
No treatment (control, after one day's accumulation)	Gill	33846±299	479
	Foot	4370± 45	928
	Gonad (viscera)	12289±111	995
	Digestive diverticula	17682±160	964
EDTA treatment	Gill	6222± 60	184
	Foot	1646± 19	540
	Gonad (viscera)	8603± 86	691
	Digetsive diverticula	8848± 89	885

Remarks; The experiment was carried out on the 26th–28th October 1954. Water temperature was adjusted to 16°C.

Discussion

The aim of this study was to investigate the picture of the calcium turnover in the freshwater fish and shellfish, particularly with reference to the biological calcification. For the illustration of the radioactivity, we used the specific activity (counts per minute per 1mg Ca in the sample) and the $C_{Calc.}$ (calculated count, counts per minute of Ca^{45} in 1g dry tissue at 0mg/cm^2 of sample thickness). In the tissues containing a small quantity of the total calcium such as the viscera and muscle (in the case of the goldfish) or the foot and adductor muscle (in the case of the pond mussel), they show higher specific activities than those of the other tissues with much inert calcium, although the net uptake of Ca^{45} in the former are smaller than in the latter. Therefore, the specific activity of a tissue is not always proportional to the corresponded $C_{Calc.}$ value.

In the goldfish, the calcified tissues such as fins, bones and operculum concentrate much calcium, while in the shellfish, the overwhelming great quantities of calcium are concentrated in the shell, and next the gill concentrates about one thirds calcium of the former, but the other organs have low calcium contents. (Table 3). The higher calcium contents of the prussian carp than the goldfish show that the calcification of the former are more advanced. The accumulation of Ca^{45} in the animal is carried out by adsorbing Ca^{45} (exactly speaking, by getting large quantities of Ca^{45} with very small quantities of Ca^{40}) and by disappearing Ca^{40} (including a very small quantities of Ca^{45}) simultaneously and the radioactive equilibrium is established between a tissue and a medium after a given interval.

The radioactive equilibrium is not a static equilibrium but a dynamic one.

The reverse is true for the disappearance of Ca^{45} from the tissues. The comparison of the accumulation curves of the goldfish and the pond mussel (Figures 2 and 3), seems to show that the shellfish accumulate much more calcium than the fish apparently. As the cause of this difference, the following factors must be considered;

(1) The difference of the moisture contents of both species.

The average moisture contents of both species are about 71 per cent for the fish and 85 per cent for the shellfish. This causes the rise of $C_{Calc.}$ values of the shellfish about two fold.

(2) The difference of the total calcium contents of both species.

The tissues of the goldfish have more inert calcium than those of the shellfish generally. This causes the lowering of the specific activities and $C_{Calc.}$ values of the fish tissues due to the self absorption of the radioactivity.

(3) The difference of the efficiency of the counter.

The counter GM 131 was only used for the accumulation of the goldfish. Its efficiency is about 90 per cent to that of the counter GM 132 for the same sample. This difference causes the rise of $C_{Calc.}$ values of the shellfish.

These factors are mainly responsible for the apparent discrepancy of the accumulation curves of both species.

The shellfish has the greater renewing fraction than the fish but has the smaller total calcium contents generally, therefore, exchangeable Ca^{45} in 1g wet tissues until the equilibrium do not differ so remarkably. The data of the exchangeable Ca^{45} in 1g wet tissues until the equilibrium for both species coincide with the pictures in Figures 2 and 3. From the Tables 7 and 8, the ratio (inert Ca : dynamic Ca) were calculated for both animals. (Table 17).

Table 17. Ratio of inert calcium versus dynamic calcium.

Goldfish		Pond mussel	
Gill	170:1	Mantle edge	89:1
Operculum	1623:1	Mantle interior	55:1
Fins	900:1	Gill	270:1
Bones	2400:1	Foot	41:1
Viscera	28:1	Gonad (Viscera)	231:1
Muscle	1100:1	Digestive diverticula	48:1
Scales	618:1	Adductormuscle	54:1
		Sshell	3330:1

In the goldfish, the disappearance of Ca^{45} proceed rapidly at the initial stage, and the disappearance rate falls gradually owing to the adsorption or the recrystallization of Ca^{45} in the tissues. When we plot the specific activities of the tissues on the logarithmic scale and the time of disappearance on the

arithmetic scale, the curves are not linear and non-reciprocal with the accumulation curves of the corresponded tissues. A part of Ca^{45} still remained in the tissues and could not be eluted out exhaustively. Although smooth disappearance curves are obtained as in Figures 4 and 5, the real pictures would be more zigzag curves, if we can measure the changes of the radioactivity during the very short time increments. In the pond mussel, the disappearance of Ca^{45} proceed more slowly than the fish. The half time and the turnover time for the shellfish is longer than those of the fish, and the turnover rate of the fish is somewhat higher than those of the shellfish. (Tables 13 and 14).

Jodrey (2) pointed out that in the oyster, *Crassostrea Virginica* GMELIN, the accumulation of Ca^{45} in the mantle edge reached to the equilibrium after four hours and the mantle edge renewed 0.023mg Ca (corresponded 2.4 per cent of its total calcium) during the turnover time of 24 minutes. Therefore, the turnover of the mantle edge of oyster is 0.06mg Ca/hour/1g fresh mantle edge. The turnover rate of the mantle edge of the oyster is two fold to that of the mantle interior, while the former has twice as much total calcium as that of the latter, the turnover time for renewing fraction is the same. About 1.34mg calcium is renewed in the whole mantle for a day.

In the case of the pond mussel, it is very interesting that the mantle interior showed higher total calcium and higher turnover rate than the mantle edge, although the reason is not yet explained. The turnover rate of the mantle in the pond mussel is about 0.001mg Ca/hour/1g wet mantle (1/60 of the oyster mantle) and about 0.04mg calcium is renewed in the whole mantle for a day (1/30 of the oyster mantle). The turnover rate of the gill in the pond mussel is 0.0025mg Ca/hour/1g wet gill (1/24 of the oyster mantle) and about 0.230mg calcium is renewed in the whole mantle for a day (1/6 of the oyster).

The calcium content of sea water is about 400mg/l and that of the pond water used was about 10mg/l (1/40). It may be probable that the difference of the Ca contents in various environments has an important effect on the calcium turnover of the animals living therein.

Above data indicate that the calcium turnover of the freshwater shellfish is less active than that of the sea water shellfish.

If we calculate from the turnover rate (Tables 13 and 14) the net uptake of calcium for a day in the goldfish and the pond mussel, the following results are obtained. (Table 18).

The results in Table 18 show that the net uptake of calcium in the freshwater fish ahd shellfish are almost in the same order, although the latter is somewhat higher than the former.

Wilbur, Jodrey (1) measured the shell formation of *Crassostrea Virginica* GMELIN and reported that from the average radioactivity per unit area of the inner shell surface, the quantity of $CaCO_3$ depositing within a month are

Table 18. Comparison of the net uptake of calcium for a day in the goldfish and in the pond mussel.

Goldfish		Pond mussel	
Tissues	Net uptake of Ca.#	Tissues	Net uptake of Ca.◎
Gill	0.033 mg (0.602)*	Mantle edge	0.023 mg
Operculum	0.024 (0.049)*	Mantle interior	0.013
Fins	0.025 (0.080)*	Gill	0.230
Bones	0.006 (0.018)*	Foot	0.005
Viscera	0.013 (—)*	Gonad (Viscera)	0.012
Muscle	0.018 (0.022)*	Digestive diverticula	0.045
Scales	0.028 (0.130)*	Adductor muscle	0.003
		Shell	0.800△
Total uptake	0.147 mg (0.914)*		0.331 mg (1.131)

Remarks : * Figures in the parentheses are obtained on the assumption that the initial rapid rate of disappearance (from 0h to 3h) being held during the experiment.
These figures indicate the net uptake of calcium in the intact tissues of 10g goldfish for a day.
◎ These figures indicate the net uptake of calcium in the intact tissues of 24.6g pond mussel (shuckled body weight 11.7g) for a day.
△ Calculated approximately from the renewed Ca^{45} in 1g intact shell in Table 9B (0.115mg) and from 7.02g of the shell weight.

calculated to be 0.92g. If we adopt the value of 0.8mg Ca/day in the case of the pond mussel shell, the quantity of $CaCO_3$ depositing for a month is 0.06g (1/15 deposition of the oyster shell).

The decontamination of Ca^{45} with EDTA is considerably more efficient than the sole immersion into the non-radioactive medium. But the exhaustive decontamination from the tissues is practically difficult even with EDTA treatment. The decontamination from the living animals with EDTA seems to be more efficient than the post-mortem decontamination.

Summary

The calcium turnover of the freshwater fish (common goldfish, *Carassius auratus* L.) and the shellfish (pond mussel, *Anodonta lauta* MARTENS) was studied.

The accumulation and disappearance curves of Ca^{45} in the tissues of the animals are illustrated in Figures 2, 3, 4 and 5.

The disappearance curves are not reciprocal with the corresponded accumu-

lation curves owing to the adsorption and recrystallization of Ca^{45} in the tissues.

The degree of the calcification is proportional to the amount of inert calcium in the tissues (calcium reservoir), i.e., the fish concentrate calcium in the so-called calcified tissues (bones, scales, fins, operculum) and the shellfish concentrate it in the shell and gill. Except the shell and gill, the shellfish has generally poorer calcium reserve than the fish. The calcium turnovers are also active in the calcified tissue for the fish and in the shell and gill for the shellfish. The calcium turnover of the freshwater animals are less active (one several tenth) than that of the seawater shellfish (oyster). Total net uptake of calcium in the shellfish (pond mussel) is somewhat higher than that of the fish. (goldfish).

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