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STUDIES ON THE FILTRATION, FEEDING AND EXCRETION RATES IN *PERNA VIRIDIS, MARCIA COR* AND *CRASSOSTREA GRYPHOIDES* (MOLLUSCA: BIVALVIA) USING P^{32} LABELLED *ANKISTRODESMES*

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ABSTRACT: The study deals with a series of experiments to investigate feeding and excretion in three species of bivalves: *Perna viridis* (Linné), *Marcia cor* (Sowerby) and *Crassostrea gryphoides* (Gould) from Manora Channel, Karachi. Bivalves were fed with suspensions of *Ankistrodesmes* labelled with P^{32}. These animals showed a considerable variation in the average filtration rates depending upon species and the body length. Exceptionally high content of the P^{32} introduced with *Ankistrodesmes*, got excreted as pseudofaeces and faeces within first three days following its absorption as a meal. The assimilated P^{32} is partly released as faecal material and its major proportion is directly transferred to the solution. As expected the gonad and kidney are the main organs found responsible for excretion as compared to other body parts. Although, the assimilated P^{32} is mostly concentrated in the digestive glands, the results also show a significant presence of P^{32} in the gonads. Accumulation of P^{32} was the least in the foot.

KEY WORDS: Filtration - feeding - excretion - bivalves - P^{32} labelled *Ankistrodesmes*

INTRODUCTION

The rates of filtration in bivalve molluscs get impaired by pollutants. Therefore, these rates have been widely accepted as a toxicity index (Abel, 1976). A number of studies have appeared in the literature in which investigations pertaining to clearance rate of marine mussels inhabiting different coastal waters of the world have been published (Rice and Smith, 1958; Allen, 1962; Abel, 1976; Mathews, *et al.*, 1984). The shores of Karachi, as other sea shores of the world, are facing an immense threat of ever increasing pollution due to human activities. Marine mussels are abundant in Karachi coastal waters and the literature is devoid of any report on the filtration rates of these very appropriate biological indicators of pollution.

For these studies tracer methods have been widely used and proved to be suitable. Further to that radionuclides have been effectively used in determining the routes and rates of distribution of food in various organs of the body, as the method causes minimum disturbance to the animal (Allen, 1962). In the present study attempt was made not only to investigate the feeding and excretion rates of three species of bivalve molluscs: *Perna viridis, Marcia cor* and *Crassostrea gryphoides*, but also to study the distribution pattern of the food in various body organs.

MATERIALS AND METHODS

The samples were collected from Manora Channel, Karachi, between June-September, 1987 and similar sized specimens were selected for the experiments.

Ankistrodesmes was grown in approximately 1000 ml of culture media to which 5 millie curie of carrier free ortho phosphate in dilute hydrochloric acid was added. Radioactive ortho phosphate having beta emitter P^{32} incorporated in it, was obtained

from PINSTECH, Islamabad. Six to ten days after inoculation, the culture was used for experimental purpose, when at least 90% P^{32} was taken up by the cells. The suspension used for the three species contained between 10-60 million cells/litre. In order to obtain maximum filtration rate (Rice and Smith, 1958), the cell number was changed with the shell length of animal under study. These suspensions were obtained by diluting the culture with seawater which was filtered through glass wool, prior to this addition. Ankistrodesmes cell counts were made using a Thomazeiss hemocytometer. The suspensions were stored in glass bowls covered with sheet of glass to prevent splashing of the radioactive suspension and also to keep out the dust and minimize the evaporation of the liquid. To ensure adequate oxygen supply and to keep *Ankidstrodesmes* floating healthy looking animals, the ones which could close their shells rapidly, were selected for study. First the shells of these animals were cleaned off any sediment and attached organisms and then the animals were transferred to the filtered seawater and kept therein for at least five days before being introduced to the *Ankistrodesmes* culture. This was done to ensure that the mantle cavity was clear of collected material and the gut was mostly free of faeces. The temperature at which the experiments were carried out varied between 30° to 35°C. The suspension (0.5ml) was counted on planchettes for its P^{32} activity at various intervals of time before and after the introduction of animals. Thereafter, the animals were removed, washed and placed in filtered sea water where they remained for 24 hours. The animals were then removed, washed and again placed in filtered sea water. The procedure was repeated till the tissue analysis was carried out. Each time the animal was transferred to fresh sea water, the accumulated faeces and pseudo faeces were pipetted out for counting on a Geiger-Muller counter (GM counter).

At the end of experiment the animal was dissected under binocular microscope and kidneys, gonads, digestive glands, gill palp, mantle and foot were transferred to the planchettes and probed for P^{32} activity on a GM counter. The tissues were thinly spread to minimize self absorption of the radiation. All counts were corrected for background and P^{32} activity in solution.

RESULTS AND DISCUSSION

FILTRATION:

Results of the experiments on the rates of removal of *Ankistrodesmes* for the three animals studied are being shown in figures 1-4, the results are not different from those reported earlier (Rice and Smith, 1958; Jorgensen, 1960; Allen, 1962). Initial lag in the curves, as reported by Allen (1962) is due to the time taken by the animal for acclimatisation. The data reveals that the filtration rates of *M. cor* are low as compared to those of *P. virdis* and *C. gryphoides*. The filtration rates of *C. gryphoides* fall into two groups. The filtration rate of smaller animals (shell length 35-46 mm) ranges from 223 to 652 ml/hr. The larger animals (shell length 100- 110 mm) have shown a higher range, 895-1535 ml/hr. A similar observation was reported by Young (1926). The filtration rate of *P. viridis*, having smaller shell length (38-59 mm) are lower than *M. cor*, which has comparatively larger shell (shell length 50-59 mm) (Table I). The filtration rates have progressively enhanced toward the end of experiment: this may

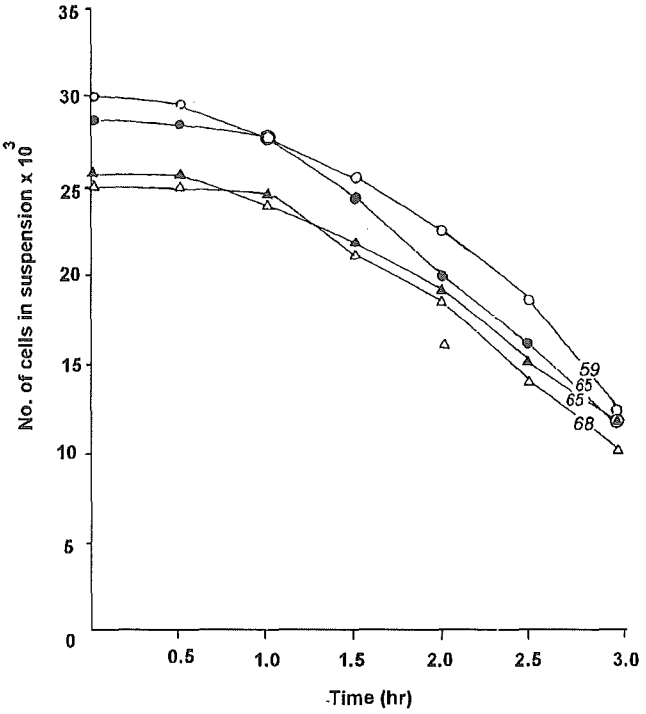

Fig.1. Rates of removal of *Ankistrodesmes* from suspension by *Marcia cor*. Italic numerals indicate shell length in mm.

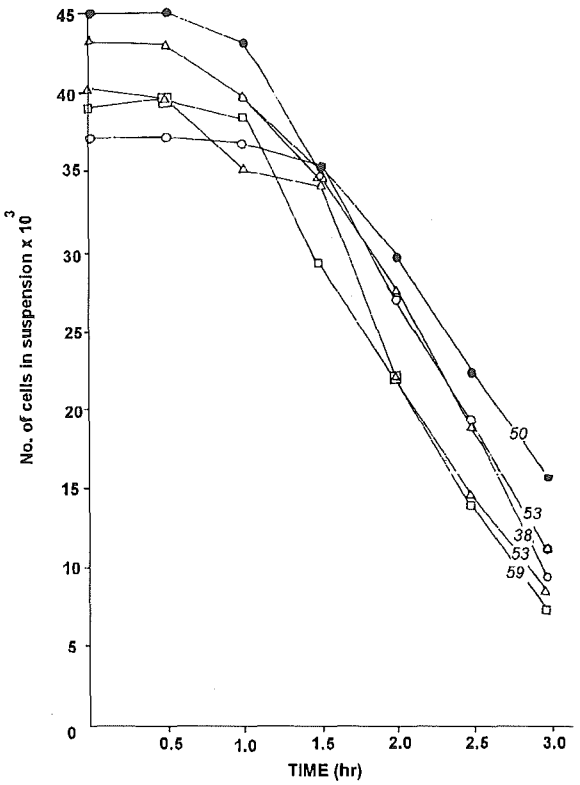

Fig.2. Rates of removal of *Ankistrodesmes* from suspension by *Perna viridis*. Italic numerals indicate shell length in mm.

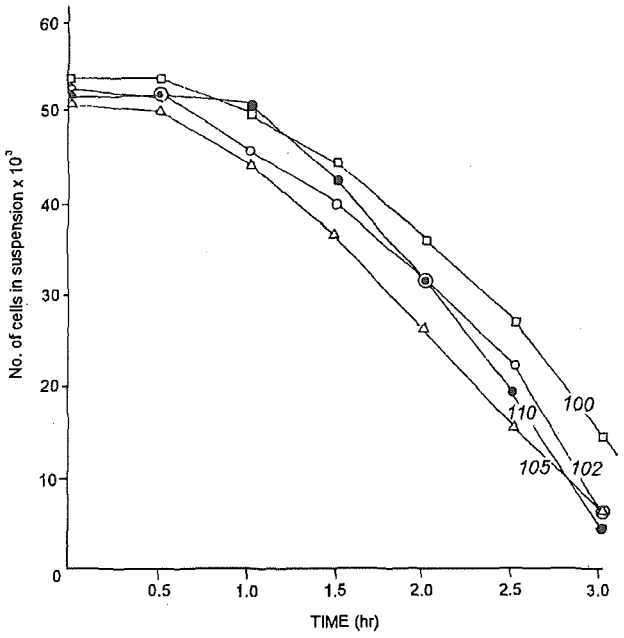

Fig.3. Rates of removal of *Ankistrodesmes* from suspension by *Crassòstrea gryphoides* (larger in size). Italic numerals indicate shell length in mm.

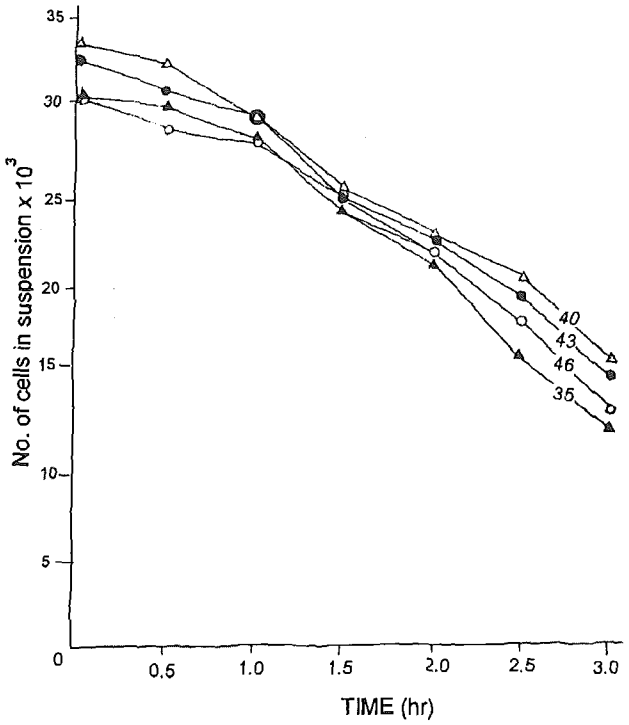

Fig.4. Rates of removal of *Ankistrodesmes* from suspension by *Crassostrea gryphoides* (smaller in size). Italic numerals indicate shell length in mm.

Table I. Filtering rates with shell length of various bivalves.

Species	Shell length mm	Filtration rates, ml/hr
Marcia Cor	50 - 68	492 - 578
Perna Viridis	38 - 59	569 - 1145
Crassostrea gryphoides	35 - 46	223 - 652
Crassostrea gryphoides	100 - 110	895 - 1535

be, as reported by Morton (1956), due to a fall in the density of *Ankistrodesmes* in supernatant.

The results provide substantial support to the suggestion that filtration rates are dependent upon body size and the surface area of the gill. *Crassostrea gryphoides* and *P. viridis* have shown high filtration rates, owing to their specific habitat. *Crassostrea gryphoides* is fixed to the rocks and *P. viridis* is attached to ship anchors in open sea. They are more exposed to currents which subject them to larger quantities of relatively clear water (Morton, 1956). In contrast to these, *M. cor* burrows in mud, having a turbid environment and accordingly a fall in filtration rate is observed. The filtration rates are thus a direct function of the pollution density of the particulate matter in the marine environment. Accordingly a fall in filtration rate is likely to reflect upon the pollution of marine waters.

EXCRETION

Faeces and pseudofaeces: Faeces and pseudofaeces excreted by the animal in the *Ankistrodesmes* suspension of seawater were transferred to planchettes and its P^{32} content was subsequently estimated through radioactive assay. The results obtained are detailed in Tables I to IV. It is observed that the animals lose more of their P^{32} activity through faeces and pseudofaeces in the commencing stage of the experiment. Towards the end of the experiment soluble form of expulsion is favoured. These results agree to the earlier work of Morton (1956).

Very little faecal material was collected for *M. cor* when it was transferred to fresh filtered seawater. However, *P. viridis* and *C. gryphoides* were found to exhibit larger faecal excretions.

The results also show that most of the excretion (75-90%) gets completed within 2 days following the end of the feeding and the remainder is released on the third day. The earlier excretion by *C. gryphoides* is green brown in colour, suggesting the presence of undigested *Ankistrodesmes*. The faecal matter excreted later is brown. The excretions from *M. cor* were also brown.

Estimates of the percentage of P^{32} taken up from the suspension and released as faecal material varies from 2-14% for *C. gryphoides*, 4-9% for *M. cor* and 3-13% for *P. viridis*.

The possible excretion of P^{32} in the form of pseudofaeces and faeces was also investigated by placing them in filtered seawater. *Marcia cor* and *P. viridis* did not

Table II: Excretion of P^{32} in faeces and pseudofaeces in *Marcia cor*

Animal No.	CPM present in suspension after 24 hrs	Calculated CPM of P^{32} extracted by animal (h)	Time in filtered seawater	Faecal CPM	Cumulative % of total P^{32}
1	5600	24,400	2	0	0.00
			26	1000	4.09
			50	490	6.10
			74	90	6.47
2	3150	21,850	2	0	0.00
			26	900	4.11
			50	205	5.05
3	6700	21,800	2	0	0.00
			26	1200	5.50
			50	750	18.94
			74	100	9.40
4	3700	22,200	2	0	0.00
			26	600	2.70
			50	300	4.05
			74	40	4.23

Table III: Excretion of P^{32} in faeces and pseudofaeces in *Perna viridis*

Animal No.	Time in algal suspension (h)	CPM present in suspension after 24 hrs	Calculated CPM of P^{32} extracted by animal (h)	Time in filtered seawater	Faecal CPM	Cumulative % of total P^{32}
1	23.00	4200	40,800	2	0	0.00
				26	3120	7.64
				50	300	8.38
				74	78	8.57
2	24.00	3900	39,500	2	0	0.00
				26	1380	3.49
				50	500	4.75
3	25.00	3500	36,600	2	0	0.00
				26	3000	8.19
				50	760	10.27
				74	70	10.46
4	26.30	3250	33,950	2	0	0.00
				26	2850	8.39
				50	1250	12.07
				74	320	13.01
				98	59	13.19
5	24.00	4450	34,450			

Table IV: Excretion of p^{32} in faeces and pseudofaeces in *Marcia cor*

Animal No.	CPM present in suspension after 24 hrs	Calculated CPM of P^{32} extracted by animal	Time in filtered sea water (h)	Faecal CPM	Cumulative % of total P^{32}
1	4,000	26,000	2	0	0.00
			26	1150	4.42
			50	500	6.73
			74	250	7.69
			100	150	8.26
			124	30	8.38
2	6,100	27,800	2	0	0.00
			26	2000	7.19
			50	950	10.61
			74	100	10.97
3	6,500	26,300	2	0	0.00
			26	950	3.61
			50	700	6.27
			74	320	7.49
			100	80	7.79
4	7,900	22,500	2	0	0.00
			26	1150	6.66
			50	820	10.31
			74	430	12.22
			100	60	12.48

show any activity, even after 72 hours. The samples from *C. gryphoides* excretions showed a very low count rate of 30 counts per minute (CPM).

P^{32} in solution: A small content of P^{32} has been excreted by the animal in soluble form as urine. The quantity of P^{32} discharged with urine was less as compared to its release along faecal material. As the animal got starved i.e. after a period of about two days following its removal from *Ankistrodesmes* suspension and introducing to fresh seawater 7- 12%, of the total P^{32} excreted, got released as soluble form. The results do not show any relationship between the quantity of P^{32} filtered off and the rate of its release into solution.

DISTRIBUTION OF P^{32} IN THE BODY TISSUES:

The food distribution was determined after 2-5 days of feeding, by dissecting individuals of each species under investigation. The results are shown in the Tables V to X, and they conform to the patterns of P^{32} distribution in other bivalves (Allen, 1962). An estimation of the total P^{32} execreted, by the species under investigation, was carried out by calculating the difference between the original and final count rates of the suspension. The absolute uptake of P^{32} by the animal was found to be independent of radiotracer concentration in solution. The faecal counts suggest that

from 70 to 90% of the total P^{32} activity is assimilated by the animal. The results are indicative of the fact that absorption of P^{32} is taking place in digestive diverticula compared to its absorption in other body tissues. The observations thus clearly demonstrate that digestive diverticula is the main storage organ.

Table V: Distribution of P^{32} in *Marcia cor*

Different organs in animal body	CPM per .02 g in different tissues of body					Estimated % of P^{32} extracted by different tissues of animals			
	Control	1	2	3	4	1	2	3	4
Digestive gland	8	2000	4500	3000	2900	9.29	24.93	15.74	15.74
Gonad	4	990	1350	1160	1080	4.60	7.47	6.08	5.78
Kidney	7	1050	700	900	820	4.87	3.87	4.72	4.39
Siphon	3	340	220	420	190	1.57	1.21	2.20	1.01
Mantle epithelium	-	500	370	200	210	2.32	2.04	1.04	1.12
Adductor muscle	-	50	47	24	70	0.23	0.26	0.12	0.37
Palp	4	250	400	310	180	1.16	2.21	1.62	0.96
Gill	5	420	50	190	160	1.95	0.27	0.99	0.85
Foot	-	40	29	30	40	0.16	0.13	0.13	0.21

Table VI(a): Distribution of P^{32} in *Crassostrea gryphoides*

Different organs in animal body	CPM per .06 g in different tissues of body					Estimated % of P^{32} extracted by different tissues of animals			
	Control-1	1	2	3	4	1	2	3	4
Digestive gland	20	3350	2990	2080	2260	12.88	10.75	7.90	10.04
Gonad	18	1920	1390	970	1080	7.38	5.00	3.68	4.80
Kidney	10	1000	1700	1800	1940	3.84	6.15	6.84	8.62
Siphon	-	-	-	-	-	-	-	-	-
Mantle epithelim	7	910	750	840	890	3.50	2.69	3.19	2.94
Adductor muscle	5	160	180	120	170	0.61	0.64	0.45	1.75
Palp	4	360	900	610	570	1.38	3.23	2.31	2.53
Gill	10	800	1000	740	590	3.07	3.59	2.81	2.62

Table VI(b): Distribution of P^{32} in *Marcia cor*

Different organs in animal body	CPM per .06 g in different tissues of body					Estimated % of P^{32} extracted by different tissues of animals			
	Control-2	5	6	7	8	5	6	7	8
Digestive gland	8	7000	6100	3800	6400	16.81	4.52	8.71	13.61
Gonad	7	4300	3000	2250	3200	10.36	7.14	5.16	6.80
Kidney	4	5000	4100	1950	1500	12.04	9.76	4.47	3.19
Mantle epithelium	5	1500	1090	1200	1500	3.61	2.59	2.75	3.19
Adductor muscle	-	300	200	180	500	0.72	0.47	0.41	1.06
Palp	4	1000	1500	1000	450	2.40	3.57	2.29	3.08
Gill	4	790	1590	600	2000	1.90	3.78	1.37	4.25

Table VII: Distribution of P^{32} in *Perna viridis*

Different organs in animal body	CPM per .04 g in different tissues of body						Estimated % of P^{32} extracted by different tissues of animals				
	Control	1	2	3	4	5	1	2	3	4	5
Digestive gland	9	6000	5300	3000	1950	4450	13.33	12.21	7.48	5.24	11.35
Gonad	4	1350	950	700	650	1000	3.00	2.18	1.74	3.25	2.55
Kidney	5	1500	2000	900	1000	2150	3.33	4.06	2.24	2.68	4.48
Siphon	2	500	190	450	200	650	1.11	0.43	1.12	0.53	1.65
Mantle epithelium	-	310	1000	1350	600	1100	0.68	2.30	3.34	1.77	2.80
Adductor muscle	-	100	150	50	60	200	0.22	0.34	0.12	0.16	0.50
Palp	20	540	600	760	470	900	1.22	1.38	1.89	1.26	2.29
Gill	-	750	970	450	195	1150	1.66	2.23	1.12	0.52	2.93

Table VIII: Content of P^{32} in the body tissues of *Perna viridis*

Different organs in animal body	CPM in whole organ of *Perna viridis* per gram; shell length in parenthesis					
	Control (54mm)	1 (50mm)	2 (53mm)	3 (53mm)	4 (38mm)	5 (59mm)
Digestive gland	20/.06	6000/.04	-	5000/.0	62500/.04	8000/.06
Gonad	7/.05	1350/.05	1600/.05	-	-	-
Kidney	10/.05	2500/.04	2000/.05	1600/.05	-	-
Siphon	5/.08	1150/.08	400/.10	900/.11	1260/.05	00/.11
Mantle epithelium	-	700/.11	600/.11	2700/.13	2000/.05	200/.10
Adductor muscle	-	300/.11	460/.12	1200/.08	1200/.04	850/.07
Palp	12/.06	1000/.05	900/.06	1200/.08	1200/.04	350/.10
Gill	4/.07	1400/.05	1700/.10	900/.09	470/.04	350/.10

Table IX: Content of P^{32} in the body tissues of *Marcia cor*

Different organs in animal body	CPM in whole tissue of *Marcia cor* per gram; shell length in parenthesis.				
	Control (50mm)	1 (59mm)	2 (68mm)	3 (65mm)	4 (55mm)
Digestive gland	20/.08	4500/.04	7000/.11	6090/.09	5000/.09
Gonad	21/.05	2700/.06	2800/.04	2050/.04	2300/.04
Kidney	19/.04	1300/.03	1000/.05	800/.03	1300/.03
Siphon	6/.11	6000/.12	650/.13	500/.11	700/.13
Mantle epithelium	4/.10	950/.12	980/.13	800/.10	600/.10
Adductor muscle	8/.20	150/.15	175/.22	100/.18	130/.19
Palp	6/.04	700/.05	300/.07	405/.06	300/.07
Gill	7/.09	700/.08	300/.07	405/.06	300/.07

Table 10: Contents of P^{32} in the body tissues of *Crassostrea gryphoides*

Different organs in animal body	CPM in tissues of *Crassostrea gryphoides* per gram; shell length in parenthesis									
	Control-1 (43mm)	1 (46mm)	2 (40mm)	3 (43mm)	4 (35mm)	Control-2 (102mm)	5 (102mm)	6 (100mm)	7 (105mm)	8 (110mm)
Digestive gland	30/.05	3350/.07	2990/.06	3100/.07	2260/.05	30/.07	9500/.10	7000/.08	4300/.10	9000/.11
Gonad	20/.06	2500/.10	1680/.08	1250/.08	1080/.08	20/.09	6000/.08	3800/.07	3200/.11	4100/.10
Kidney	15/.02	1000/.04	1700/.03	1800/.04	1940/.02	15/.07	5000/.05	4100/.04	1950/.05	1500/.06
Mantle epithelium	10/.08	1500/.12	1000/.10	1000/.07	1000/.07	10/.12	2500/.11	2000/.10	1650/.10	- /.11
Adductor muscle	-	250/.20	220/.16	190/.14	250/.10	-	650/.20	250/.16	300/.20	- /.21
Palp	19/.06	360/.06	900/.06	610/.06	570/.06	16/.08	1500/.07	1350/.06	1200/.07	- /.08
Gill	13/.12	1500/.10	1200/.08	850/.08	900/.08	-	1200/.12	1300/.10	2000/.09	- /.10

The second highest concentration of P^{32} was found in kidney and gonad because it was a possible site for phosphorus excretion (Allen, 1962) and gonad being the site where phosphate ions are probably absorbed (Marshall and Orr, 1955). The appearance of P^{32} in the body suggests that some of the tracer entered across the gill palps, mantle and to lesser extent into adductor muscles. The uptake of P^{32} by the digestive glands in *C. gryphoides* and *P. viridis* is more as compared to *M. cor.* The concentration P^{32} also seems to be related to the age of the animal as do the filtration rates. There is a considerable difference between the concentration of P^{32} in various body organs of *P. viridis* having smaller shell length and the others having larger shell length (Table I). Though the concentration of the radiotracer in the adductor muscles and siphon is much lower than in other tissues (Tables V-X), they still store considerable amounts of phosphorus as they carry a major share of the animal weight.

Results from control experiments with *C. gryphoides* which is the largest of the other two, show that a larger amount of P^{32} was stored by this animal and thus the suggestion that the P^{32} absorption is related to body size is strengthened.

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REFERENCES

Abel, P.A. 1976 Effect of some pollutants on the filtration rate of *Mytilus*, *Marine Pollution Bulletin.* 7: 228-231.

Allen J.A. 1962. Preliminary experiments on the feeding and excretion of bivalves using *Phaeodactylus* labelled with P^{32}. *Journal of the Marine Biological Association, U.K.* 42:609-623.

Jorgensen, C.B. 1960. Efficiency of particle relation and rate of water transport in distributed lamlibranchs. *Journal Conseil Pour l'Exploration De La Mer* 26:116-94.

Marshall, S.M. and A.P. Orr. 1955. On the biology of *Calanus*, food uptake, assimilation and excretion. *Journal of the Marine Biological Association U.K.* 34: 525-529.

Mathews. R. and N.R. Menon. 1984, Filtration in *Perna viridis* and *Meretix Casta* under heavy metal stress, *Mahasagar-Bulletin of the National Institute of Oceanography* 17(3): 183-186.

Morton. J.E. 1956. The tidal rhythm and action of the digestive system of the lamellibranch *Lasaea rubra. Journal of the Marine Biological Association,* U.K. 35: 563-586.

Rice, T.R. and R.J. Smith. 1958. Filtering rates of the hard clam *Venus mercenaria* determined with radioactive Phytoplankton. *Fisheries Bulletin* 58: 82-73.

Young, C.M. 1926. Structure and physiology of feeding and digestion in *Ostrea edulis. Journal of the Marine Biological Association, U.K.* 14: 386-395.