THE ELIMINATION OF FOREIGN PARTICLES INJECTED INTO THE HAEMOCOELS OF SOME PROSOBRANCHIATE GASTROPODA

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INTRODUCTION

This is one of a series of papers dealing with the elimination of foreign, particulate material from the body fluids of invertebrate animals. In the past such studies have not been carried out systematically; a wide variety of substances has been introduced by different workers into different invertebrates and little or no attempt has been made to ensure that the experimental animals are kept under comparable or even controlled conditions. It is thus not possible to compare the results gained by different workers and there is virtually no information available on the rate of elimination of particles or the factors affecting it. The only exception of note appears to be the meticulous work of Feng (1962, 1965, etc) on the oyster, *Crassostrea virginica*. Also, there are groups of animals which have been completely overlooked as far as the elimination of foreign particles is concerned.

In the present series of investigations, the radio-opaque dye "Thorotrast" is being invariably used as the injected material; the advantages of this material over the more usual carmine or indian ink have been detailed elsewhere (Brown 1964, Brown & Brown 1965). The amount of Thorotrast injected is noted in all cases and is roughly proportional to the tissue weight of the animal; the temperature is kept constant and is as far as possible consistent with temperatures experienced by the animals in the field. Where possible, the animals are fed immediately before the experiment commences, after being kept for several days under the controlled conditions. Attempts are made to ensure that their opportunities to feed during the period of the experiment are similar to those offered under natural conditions. These factors are considered to be important as it is thought that the rate of elimination of the material may be greatly influenced by temperature and by the nutritional state of the individual. In this way it is hoped to gain comparable data from different groups of invertebrates and to reduce some of the gaps in our knowledge of the subject. The technique has thus far been applied to the sandy-beach prosobranch, *Bullia* (Brown & Brown 1965), the terrestrial pulmonate, *Helix* (Brown 1967a) and the holothurian, *Cucumaria* (Brown 1967b). Work is also in progress on the sipunculid, *Golfingia*, and the tunicate, *Ciona*.

As in the case of other invertebrate animals, small solid particles in the body fluids of gastropod molluscs are normally phagocytosed by macrophagic amoebocytes which then migrate with them and leave the body by various well-defined routes. In the pulmonate, *Australorbis*, the main migratory pathway appears to be through the mantle epithelium and adjacent surfaces (Tripp 1961), while in *Helix* the reproductive tracts are heavily involved in addition to the gut and mantle epithelia (Brown 1967). In the prosobranch, *Bullia*, on the other hand, the chief pathway is through the wall of the heart, into the pericardial cavity and thence through the renopericardial canal into the lumen of the kidney, from which the laden cells escape from the body by way of the nephropore (Brown & Brown 1965). Migration through

the mantle epithelium also takes place in *Bullia* but, although it represents a much shorter route, this pathway appears to be of relatively minor importance. Some migration also takes place through the tissues of the kidney and into its lumen from the surrounding blood spaces and laden amoebocytes may also join the chief migratory stream by entering the pericardial cavity through the pericardial wall. The digestive gland, gut, gill and reproductive organs appear never to be involved in the elimination of foreign particles in this animal.

The present investigation constitutes an attempt to discover whether or not the routes taken by migrating cells in *Bullia* are typical of the Prosobranchiata as a whole, *Bullia* being the only genus of this group which has previously been investigated. Notes have also been made on the rate of elimination of the particles and the extent of this elimination.

MATERIAL AND METHODS

Seven species of intertidal rocky-shore prosobranchs and one infratidal form from soft substrata were selected for study. They were *Patella cochlear* Born., *Patella granularis* Linn., *Turbo sarmaticus* Linn., *Oxystele variegata* (Anton), *Burnupena cincta* (Bolten), *Thais dubia* (Krauss), *Littorina knysnaensis* Philippi and the predominantly infratidal *Xenogalea zeylanica* (Lam.). Thus representatives of all three orders of the Prosobranchiata were included in the investigation. The rocky-shore snails were collected from Dalebrook and from Kalk Bay, on the False Bay coast of the Cape Peninsula, while *Xenogalea* was obtained from shallow water off Fish Hoek Beach. All individuals were thus collected within a few miles of one another in an unpolluted area. They were maintained in the laboratory at a temperature of 15C ($\pm$ 1·0); the rocky-shore snails were kept in a tank containing sea-water and stones from their natural habitat, a slope being provided so that they could crawl out of the water. The individuals of *Xenogalea* were kept separately, in a smaller tank, over a substratum of beach sand. The specimens used were large adults (of both sexes), except in the cases of *Turbo* and *Thais*, large individuals of which could not be found at the times of collecting.

Under the conditions provided, most of the species thrived and some individuals were kept alive for several weeks after the end of the experiment. The exceptions were the two species of *Patella*, which proved impossible to keep alive for longer than 8 or 9 days from the day of collection. While the individuals of the other species were maintained in the laboratory for 3 or 4 days before being injected with Thorotrast, this was clearly impracticable in the case of *Patella* and the animals had to be injected on the day of collection. Even then records could only be obtained for a maximum of 9 days and for much of this time the animals were in poor condition. A subsequent series of experiments, with *Patella granularis* only, was thus conducted on the shore, individuals being lifted from the rock, injected with Thorotrast, and then replaced in their original positions. They were finally marked with "Necol" anti-fouling paint for subsequent identification. This treatment appeared to have no serious ill-effects on them and they continued to be available when required for many weeks.

The volume of Thorotrast injected was roughly proportional to the size of the animal and was of the same ratio as that used in previous experiments on *Bullia* and *Helix*, i.e. about 0·1 cc for every 1·5 gm body weight. Approximate tissue-weights were calculated by weighing the individual to be injected and then applying average tissue-weight/whole weight ratios arrived at previously. Some of these ratios have been recorded by Brown (1960). Injection of this

material into the pedal sinus of *Patella* presented no difficulty but in the other snails gave more trouble than in the case of *Bullia*, due to the lower thresholds of retraction. Attempts to anaesthetise some of the animals before injection did not lead to marked success and the final method adopted was to inject Thorotrast into the pedal sinus directly through the operculum of the unanaesthetised, retracted animal. This technique is easily mastered after some practice.

In the initial experiments, animals were killed 3, 5, 7 and 10 days after injection and removed from their shells. They were preserved in 70% ethyl alcohol and later X-rayed by means of a diagnostic X-ray machine, the exposure being 0·03 sec. at 100 mA and 55 kV at a focus-film distance of 100 cm. In a subsequent series of laboratory experiments, using a larger number of animals (but no representatives of *Patella*) individuals were killed daily for ten days after injection and then on the fourteenth and twenty-first days. Any remaining individuals were killed and preserved at various times thereafter. In the case of the specimens of *Patella granularis*, injected on the shore, some were killed every second day for fourteen days and a last two on the twenty-first day. All the preserved animals resulting from the second series of tests were X-rayed, after removal from their shells, by means of a Siemens' diagnostic X-ray machine with enlarging facilities, the exposure being 2·0 sec. at 16 mA and 60 kV. Positive enlargements were made from some of the radiographs, while the remainder were examined in their original form, the pictures being 4 times larger than life. A few of the preserved snails were sectioned, the sections being viewed by the method of Baxter (1960).

Haemolymph was at times withdrawn from the pedal sinuses of some of the animals with the object of counting the haemocytes and assessing the effect of injections of Thorotrast on the haemocyte populations. The method used was that previously reported for *Bullia* (Brown & Brown 1965). Results are not reported in detail owing to their inconsistency, but some information on these cell-counts is given below.

RESULTS

(*a*) RADIOGRAPHS AND SECTIONS

The experiments on *Littorina* failed, largely due to the small size of the animal and the small dose of Thorotrast which it was attempted to administer. It was not possible to be sure of injecting the material into the pedal sinus; in some cases part of the material was injected into the gut while in others the final radiographs show no Thorotrast shadows at all, implying that the material may not have entered the animal. However, shadows are apparent on radiographs of all the other species examined and sections reveal the presence of thorium particles in the tissues and body fluids. As in the case of *Bullia*, all the thorium particles seen in section were confined to cells whose appearance was invariably consistent with the view that they were amoeboid haemocytes. The results may be summarised as follows:—

Patella cochlear: in all the radiographs (of animals killed 3, 5, and 7 days after injection as well as of animals which died on the subsequent two days) the most intense shadows were confined to the periphery of the limpet. They involved the mantle fringe as well as the pallial gills and were particularly dark at the distal margins of the latter. These shadows were already much in evidence by the third day but were most marked on the fifth. Animals preserved on the seventh, eight and ninth days gave radiographs showing shadows of an intensity which was, for the most part, comparable with that seen on the fifth day, but in two cases the shadows

were noticeably fainter. Neither the heart nor the pericardial cavity cast shadows on any of the radiographs and sections revealed no thorium-laden cells in the pericardial cavity though they were to be seen in the heart itself. The reproductive organs were found to be quite free of thorium-laden cells and though such cells were seen in the blood spaces around the (right) kidney, only one was observed in the lumen itself. The radiograph of one of the animals killed on the fifth day showed some involvement of part of the alimentary canal and sections revealed laden cells in the gut wall. However, the other animals sectioned gave negative results in this respect.

Patella granularis: as already noted, two sets of experiments were conducted on this species; one in the laboratory under controlled conditions which resulted in the death of the animals, and a second under uncontrolled field conditions, the health of the animals remaining good throughout the experiments. The laboratory animals (killed 3, 5, and 7 days after injection of Thorotrast) gave results very similar to those obtained with *P. cochlear*. Again the shadows involved mainly the mantle skirt and the gills, and these appeared darkest in animals killed on the third and fifth days. In section it could be seen that the blood lacunae of the mantle fringe and gills were almost choked with thorium-laden amoebocytes, while others were to be seen in the epithelia themselves. There was no involvement of the pericardial cavity or the lumen of the (right) kidney. Migration through the mid-gut wall was in evidence in section, but not sufficiently to cast shadows on the radiographs at the exposures used. Radiographs of animals killed on the seventh day showed, in some cases, rather fainter shadows but sections revealed that a considerable amount of thorium remained in the body on this day, though laden cells were no longer in evidence in the arterial system or heart.

The second series of experiments, conducted on the shore, gave similar results except that the elimination of the particles appeared to proceed very much faster. Heavy shadows involving the mantle skirt and gills were already apparent by the second day after injection and on the fourth day appeared, on the whole, somewhat fainter. They were markedly so by the sixth day and some of the individuals killed on the eighth day cast no visible Thorotrast shadows at all. In sections of one of the latter specimens, only a few thorium particles could be seen, all of them in cells which lay embedded in the mantle or gill epithelia. By the tenth day none of the animals killed cast Thorotrast shadows and sections revealed that virtually all traces of thorium had been eliminated.

Turbo sarmaticus: in animals killed 24 hours after injection the shadows were still diffuse and ill-defined, except for a single shadow involving the heart. Sections through one such animal show an accumulation of thorium-laden amoebocytes in the heart, while the arteries and the veins leading immediately into the auricle also show large numbers of such cells. By the second day the heart-shadow seen on radiographs had darkened and in some cases a faint shadow could be seen which clearly represents the pericardial cavity. By the third day the pericardial shadow was very well defined, as was a large shadow cast by the left kidney, or papillary sac. Sections through this kidney revealed masses of laden amoebocytes both in its lumen and in the blood spaces of the papilla. Regrettably it was impossible to make out in these or in subsequent sections whether amoebocytes were actually migrating across the epithelium of the papillary walls. By the fifth or sixth day, shadows were noticeably fainter and by the eighth or ninth day the only shadow which could be clearly distinguished was that cast by the left

kidney. Sections through an animal killed on the ninth day showed numerous laden cells in the blood spaces and tissues of the papillary sac, though not in its lumen. Other parts of the body were almost free of thorium. Although none of the radiographs obtained from this species gave a well-defined mantle shadow there can be little doubt that the mantle epithelium is used as a migratory pathway; sections of animals killed between the second and sixth days revealed a fair number of laden amoebocytes in the blood spaces of the mantle, often attached to or embedded in the surrounding tissue.

Oxystele variegata: the position in this animal appears somewhat complex, a large number of pathways being used by migrating cells. As in the case of *Turbo*, a major route involves the left kidney, probably through the tissues themselves and certainly from the heart via the pericardial cavity and renopericardial canal. The mantle also provides an important pathway and the gut is involved to some extent. The most interesting feature, however, is the involvement of the gill; this is intense enough to cast a well-defined Thorotrast shadow on some of the radiographs of animals killed between the third and sixth days and sections showed numerous amoebocytes in the blood-vessels of the gill and invading the gill epithelia. The gill may thus constitute an important pathway in this animal. The position is complicated by the fact that in this species there appears to be considerable variation between individuals, both in the relative importance of the different migratory routes and in the sequence of events. Thus shadows were apparent by the second or third day but, while in radiographs of some snails killed during this period it was the heart which cast the most intence, or even the only, shadow, in one radiograph the gill showed up most darkly while in another both the gill and mantle were heavily involved and the heart shadow had just begun to form. By the fourth and fifth days both the pericardium and the left kidney usually cast intense shadows but in one radiograph no pericardial shadow was seen, though the kidney is clearly involved. In another the gut is visible for part of its length and in several the gill still cast a shadow. Radiographs taken on the sixth and seventh days generally showed fainter shadows but one showed shadows which were on the whole, darker, particularly in the renopericardial region. By the tenth day the only shadows seen consistently were those cast by the heart and the left kidney, though in a few cases the pericardium continued to cast a faint shadow. The kidney shadow persisted long after the others had faded, could be seen in the radiographs of all three animals killed on the fourteenth day and was still apparent, though very faint, in one of two animals killed on the twenty-first day after injection. Sections through the latter animal confirmed that the only thorium which remained in the snail was confined to the tissues of this left kidney. Sections through an individual killed on the fifth day after injection indicated that only the structures already mentioned are important as migratory routes. The reproductive organs, digestive gland and body wall appeared to be quite free of laden amoebocytes.

Xenogalea zeylanica: As this species was the only representative of the Mesogastropoda available, other than *Littorina*, it was hoped to make a very thorough study of it. However the work was considerably hampered by the difficulty of obtaining material. Enthusiasm was also dampened by the initial experiments, which indicated that the animal was quite unexceptional as far as the elimination of foreign particles was concerned. The most important route for laden phagocytes was found to be through the wall of the heart into the pericardial cavity and from there to the exterior by way of the renopericardial canal and kidney. Laden cells

were also to be found in considerable numbers in the tissues of the kidney. The mantle cast no shadow, though sections revealed that the mantle epithelia do in fact provide a migratory pathway. The shadows cast by the renopericardial route and the tissues of the kidney were generally visible by the third day after which they darkened rapidly, becoming most intense on the sixth or seventh day after injection of the animal. They were relatively faint by the tenth day but could still be made out on the fourteenth; sections through an animal killed 14 days after injection revealed thorium particles still present in some quantity. After 21 days no shadows could be made out and the general opacity of the body was similar to that of a control animal killed at the same time. However sections through the kidney of one of the test animals showed that some thorium particles were still present in the tissues, particularly in the tissues of the kidney.

Burnupena cincta: In this species, as in *Xenogalea*, the most important migratory routes were found to be the renopericardial pathway and, probably, the tissues of the kidney. However the mantle was also sufficiently involved to cast shadows in many of the radiographs. Sections not only confirmed these routes but also showed some laden phagocytes embedded in the wall of the gut. No such cells could be found in the lumen of the alimentary canal, however, and it cannot be considered proven that the gut is really used as a migratory route. Even if it is used, it is probably of very minor importance to judge from the number of laden cells associated with it. The heart shadow took 3 to 4 days to develop and it was 6 days before the pericardial cavity was consistently involved. Within 24 hours the lumen of the kidney cast a dark shadow and the whole pathway was most intense on the seventh or eighth day. Fading proceeded slowly and the shadows were still quite strong on the fourteenth day. Of two individuals killed on the twenty-first day, one showed no Thorotrast shadows at all while radiographs of the other continued to show a faint renopericardial shadow. A single individual killed four weeks after injection cast no well-defined shadows but sections revealed that thorium particles were still present in cells embedded in the kidney tissues, though they could not be found elsewhere.

Thais dubia. The sequence of events in this species was similar to that reported above for *Burnupena*, except that no evidence pointed to involvement of the gut. In addition, on some radiographs, the gill could be made out as a faint shadow; however, while sections through the gill lamellae of such individuals showed numerous laden phagocytes in the blood spaces, no invasion of the epithelia was in evidence. The renopericardial shadows took about a week to reach maximum intensity, though the outline of the heart could be seen on radiographs taken 3 or 4 days after injection of the dye. The mantle also cast a shadow during this period but quickly faded and was not in evidence after the seventh day. Renopericardial involvement was still apparent on the fourteenth day and in some animals these shadows could still be made out in radiographs taken on the twenty-first day. Thorium particles could be discovered in individuals killed after four weeks and in a single snail killed six weeks after injection of Thorotrast.

(*b*) HAEMOCYTE COUNTS

The inconsistency of the results gained from haemocyte counts did not allow of detailed analysis and no clear picture of the effect of foreign particles on the haemocyte populations can be given. Nevertheless, certain aspects of the work may be reported here. Firstly no sample of

haemolymph withdrawn from the animals used in the present investigation gave haemocyte counts comparable with those obtained from *Bullia* (Brown & Brown 1965). In *Bullia*, haemolymph from the buccal sinuses of fresh, untreated snails kept at 15C gave haemocyte counts which ranged from 3,800 to 7,200 cells per mm^3. These figures are not particularly high for an invertebrate; yet in the present investigation no sample of haemolymph (withdrawn from the pedal sinus) had a haemocyte population exceeding 1,500 per mm^3 and the majority of counts were below 1,000. This is not entirely due to the different site of collection, for a single sample of haemolymph subsequently collected from the pedal sinus of *Bullia laevissima* gave a cell-count of 4,800 per mm^3. Thus either the haemocyte population in *Bullia* is higher than in other prosobranchs or, what would appear to be more likely, circulation in *Bullia* is more efficient so that the haemocytes tend to remain in suspension even in the sinuses and do not settle out to the extent found in the other prosobranchs examined.

TABLE 1

PATHWAYS TAKEN BY LADEN AMOEBOCYTES IN THEIR MIGRATION FROM THE BODY FLUIDS TO THE OUTSIDE OF THE ANIMAL. THE FIRST EIGHT SPECIES ARE PROSOBRANCHIATA; THE LAST TWO ARE PULMONATES, INCLUDED FOR COMPARISON.

Species	*Reno pericardial*	*Mantle*	*Gills*	*Gut*	*Reproductive organs*
Patella cochlear	—	Major route	Major route	Of very little significance	—
Patella granularis	—	Major route	Major route	Minor route	—
Turbo sarmaticus	Major route	Of some importance	—	—	—
Oxystele variegata	Major route	Important route	Possibly of some importance	Minor route	—
Xenogalea zeylanica	Only important route	Minor route	—	—	—
Burnupena cincta	Major route	Important route	—	Doubtful	—
Thais dubia	Major route	Important route	—	—	—
Bullia laevissima	Major route	Important route	—	—	—
Helix aspersa	—	Of some significance	—	Probably important	Major route
Australorbis glabratus	—	Most important pathway	—	—	—

TABLE 2

RATE AND EXTENT OF ELIMINATION OF THORIUM PARTICLES FROM 7 SPECIES OF PROSOBRANCHIATA AND 1 PULMONATE (*Helix*). THE FIRST THREE COLUMNS GIVE ASSESSMENTS OF THE TIME TAKEN FOR THOROTRAST SHADOWS TO APPEAR, REACH MAXIMUM INTENSITY AND FADE, JUDGED ENTIRELY FROM RADIOGRAPHS MADE AT A UNIFORM EXPOSURE. THE LAST COLUMN INDICATES THE DEGREE OF ELIMINATION AFTER VARYING PERIODS, JUDGED FROM SECTIONS.

Species	*Time in days*			*Extent of elimination*
	First appearance of shadows	*Darkest shadows*	*Disappearance of shadows*	
Patella granularis	1–2	3	8–9	Complete in 10–11 days
Turbo sarmaticus	1–2	3–4	10–14	?
Oxystele variegata	2–3	4–6	14–21	Virtually complete in 21 days
Xenogalea zeylanica	3	6–7	less that 21	Incomplete after 21 days
Burnupena cincta	3–4	7–8	about 21	Incomplete after 28 days
Thais dubia	3–4	6–8	about 21	Incomplete after 42 days
Bullia laevissima	4–5	7–11	30–40	Incomplete after 6 weeks
Helix aspersa	3	4–5	about 12	Very incomplete after 12 days

Haemolymph withdrawn hourly after injection of Thorotrast into *Burnupena cincta* showed a marked decrease in the originally small number of circulating macrophages and no macrophages at all could be found after five hours. Thorium particles could be seen in the blood for the first 3 hours but no free particles were found in subsequent samples. Macrophages were again seen in samples withdrawn after 24 hours.

DISCUSSION AND CONCLUSIONS

The chief aim of this investigation was to discover whether the pathways used by laden, migratory phagocytes in *Bullia* were typical of the Prosobranchiata as a whole. Results are summarised in Table 1, *Helix* and *Australorbis* (two pulmonates) being included for the sake of comparison. The only pathway used consistently, in both prosobranchs and pulmonates is through the mantle into the mantle cavity. However, the importance of this route differs from species to species. In *Patella*, if we consider the gills to be merely a specialised part of the mantle, it is the only major pathway for the elimination of foreign particles, while in the other species examined it varies from being an important route to one of relatively minor significance. Perhaps the emphasis on the mantle, and structures derived from it, in *Patella* is not altogether unexpected in view of the observation of Cuénot (1914) that particles he injected into the blood stream became localised in the pallial gills, where a large concentration of phagocytes was found to be present. *Patella* has no papillary sac, the left kidney being reduced to a small patch of tissue; several authors have commented on this fact and have suggested that the lack of phagocytic activity usually associated with the papillary sac might necessitate particles being excreted by a different route. However, while the papillary sac undoubtedly

displays phagocytic activity in some species (Fretter & Graham 1962), in the present context it is possible that the absence of an effective channel from the pericardial cavity of *Patella*, through the sac to the exterior, is of greater importance.

It is interesting to note that under these circumstances the right kidney, which provides such a channel, is not used as an alternative route. This is consistent with the fact that in the other Archaeogastropoda examined (*Turbo*, *Oxystele*) it is only the left renopericardial channel which is exploited, the right system being apparently not involved at all. In higher prosobranchs it is considered to be the left kidney which persists and this is certainly supported by the present evidence, for both in Mesogastropoda (*Xenogalea*) and in Neogastropoda (*Burnupena*, *Thais*, *Bullia*) the renopericardial pathway is the most important route taken by migrating phagocytes. The relationship between these free cells and the fixed phagocytes of the kidney is not clarified by the present investigation but, as Potts (1967) has pointed out, this relationship "recalls the equally complex relationships in the vertebrates between the monocytes and macrophages of the blood and the reticulo-endothelial system." In some prosobranchs (*Bullia*, *Turbo*, *Oxystele*) the tissues of the left kidney are clearly involved in the elimination of particles but what remains to be discovered is whether this involvement is confined to a simple migration of free phagocytes from the blood through the kidney epithelium into its lumen, or whether particles are actually picked up by the attached phagocytes and then passed on to free cells.

Other migratory routes appear to be of little importance among the Prosobranchiata; involvement of the gut is slight, if present at all, and only in the case of *Oxystele* is a non-pallial gill possibly of importance, though in several other species an initial congregation of laden cells in the blood spaces of the gill is apparent. Several potential migratory pathways appear to be utilized not at all, or to so small an extent that they were not detectable by the methods used. These include the digestive gland, the body wall covering foot and head, and the reproductive organs. That the reproductive organs could represent an important pathway is clearly demonstrated by their use in the elimination of foreigh particles in *Helix*, though the lamellibranchs, like the prosobranchs, do not seem to have exploited it (Stauber 1950).

The rate and completeness of the elimination of thorium particles differs from species to species, and also to some extent between different individuals of the same species. For convenience the relevant data have been brought together in Table 2. The figures for *Patella cochlear* have not been included in this table as the individuals were in poor condition for most of the time and the results are probably not comparable with those gained from the other species. The figures for *Patella granularis* are those obtained from the experiments conducted on the shore. In this species the elimination of thorium proceeded faster on the shore than in the laboratory but whether this was because of the poor condition of the laboratory individuals or because of higher average temperatures on the shore is not known.

In Table 2 the prosobranchs have been arranged in the order of decreasing rate of elimination of particles. The Archeogastropoda display a faster rate of elimination than do the Neogastropoda, while *Xenogalea* appears to occupy an intermediate position. There is no evidence, of course, that the condition in *Xenogalea* is typical of the Mesogastropoda as a whole. An interesting aspect of these results is the comparatively slow elimination of thorium in *Bullia*,

the Thorotrast shadows persisting for up to 40 days. This may well be a real difference but it is somewhat disturbing that the experiments on *Bullia* were conducted separately from those on the other prosobranchs; while every effort was made to ensure that the experimental conditions were identical for all species, it is perhaps possible that factors not taken into account play a part in the speed of elimination of particles. The time of year is one such factor which suggests itself. The pulmonate, *Helix*, on the other hand displays a rate which is comparable with *Turbo* and *Oxystele;* the work on this animal as well as on the prosobranchs reported in this paper was performed in summer and early autumn, while the work on *Bullia* was done in late winter.

SUMMARY

This paper reports the elimination of thorium particles injected into seven species of prosobranchiate Gastropoda, and compares the results gained with previously reported work on other species. In all cases the particles are phagocytosed by macrophages which then migrate to the exterior of the body. The mantle presents the only consistent route but more important pathways in the majority of prosobranchs involve the wall of the heart, the pericardial cavity and thence through the (left) renopericardial canal and the lumen of the (left) kidney to the nephropore; the papillary sac is also typically involved. The right renopericardial channel, where it exists, is not used as a pathway. The gut and gills are of relatively little significance as migratory routes and the reproductive organs appear not to be used at all in the Prosobranchiata. The rates of elimination of the particles in the various species are discussed.

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