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EXPERIMENTS WITH RADIOACTIVE STRONTIUM (^{90}Sr) ON CERTAIN MOLLUSCS AND POLYCHAETES

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(Text-figs. 1–5)

Introduction

The use of radioactive strontium in the study of invertebrate metabolism is as yet not extensive; it has been employed successfully by several workers on the metabolism of bone. In both vertebrate and invertebrates calcium is the more important element and strontium, if present, is only in traces, except for the aberrant group of radiolarians, the Acantharia, in which the skeleton consists almost entirely of a salt of this element. Radiocalcium would be the ideal choice for these studies of metabolism, but the radioactive isotope of calcium, ^{45}Ca, has been available up till now only in small quantities and with weak activity. Chemically, and to some extent physiologically, however, strontium replaces calcium (McCance & Widdowson, 1939). Pecher (1941*a*, *b*) has shown that in mice, rats and man the metabolism of the two elements is similar qualitatively, though there are quantitative differences: both are concentrated in the skeleton, with very little in the soft tissues, but the uptake of calcium is greater than that of strontium. Resemblances in metabolism of the two elements in vertebrates are also demonstrated by Posin (1942) and by Erf & Pecher (1940). Thus it is because of their similar behaviour, and the availability of radioactive strontium which is carrier-free, that this element has been of interest as a tracer for the study of calcium metabolism in mammals. It is also employed by Swann (1950) to detect the glands which secrete the calcareous tubes of serpulid polychaetes. Exactly how strictly it follows the course of calcium in invertebrates is not known.

Spooner (1949) studied the absorption of radioactive strontium and yttrium by marine algae: comparing the decay characteristics of specimens of water in which the alga had been kept with control specimens, he was able to estimate the amount of strontium, as apart from yttrium, which was taken up by the weed. He suggests that the extraction of yttrium from the water involves adsorption by the surface of the tissue as well as ionic exchange, both processes being important with red algae. Autoradiographs of microtome sections of *Rhodymenia* have therefore been made to follow up Spooner's suggestion: they show, as he predicted, a surface adsorption layer of ions comprised mainly of yttrium, but also with some strontium. In animals,

specially in a form like *Mytilus*, on which preliminary experiments were carried out, such adsorption detracts considerably from the value of quantitative work, for the isotopes are not only adsorbed on any glass surfaces involved in the experiment, but also on the shell of the bivalve, the byssus threads which may be secreted during the experiment, the faeces, and any particle of detritus or micro-organism in the sea water or on the surface of the body. The decay-curves of pieces of *Mytilus* shell which have been immersed in activated sea water, then washed in distilled water, placed on a microscope slide and presented to a GM4 counter, resemble those of similarly treated *Rhodymenia* fronds (Spooner, 1949): about 90% of the activity is due to yttrium which is differentially concentrated.

^{90}Sr, the radioactive strontium which was used for the present investigation, was supplied by the Radiochemical Centre, Amersham. It has a half-life of 25 years; it breaks down by β-particle-emission to ^{90}Y. The yttrium is itself radioactive and has the comparatively short half-life of 63·4 hr., changing in turn to stable zirconium, ^{90}Zr. Tests made at Amersham show that 1 mc. of the ^{90}Sr would have a total weight of strontium within the range 10–70μg.; no inactive strontium was used for the extraction process, which was carried out using lead as carrier. Therefore, when sea water is activated by this ^{90}Sr to the extent of 100μc./l. there is an addition to the water of not more than 7μg. of strontium; the doses which were employed for marine organisms were kept within this quantity unless otherwise stated. Sea water from the English Channel contains 10 mg. Sr/l.: the addition of 7μg. means, therefore, an increase of only 0·07%.

The uptake of ^{90}Sr and ^{90}Y by the tissues of certain invertebrates was studied by means of autoradiographs. These detect the distribution of comparatively minute quantities of the tracer element, and are particularly useful in studying small invertebrates which have soft bodies, since autoradiographs can be made with sections of the intact animal and the concentration of the isotope by specific cells can be followed. For the preparation of such autoradiographs animals were fixed in absolute alcohol—a fixative which is useless for the preservation of cytological detail but advantageous in that, unlike acid fixatives, it does not appear to inhibit the sensitive emulsion of the stripping film in recording tracks of the β-particles which are emitted during the decay of the tracer. After fixation the tissues are cleared in xylol, embedded in paraffin wax, and sectioned to a thickness of not more than 10μ. Sections are floated on to slides which have been previously coated with 1% gelatine solution; later the wax is removed with xylol, the tissues hydrated, washed well in distilled water and covered with the fine-grain stripping film supplied by Kodak Ltd. The sensitive emulsion of the film is thus brought into intimate contact with the tissues, and this exposure may last from a few days to several weeks; during such a period the slides are stored in a light-tight box in a refrigerator. At the appropriate time the film, still covering the sections, is

developed in amidol developer; the slides may then be placed in haemalum to stain the tissues, and the stain differentiated with 1% HCl, which, at the same time, removes it from the film stripping.

The use of ^{90}Sr concerns two tracer elements, strontium and its daughter-product yttrium; it would be an advantage to have some method by which the activity derived from the strontium intake alone might be shown on an autoradiograph. Since ^{90}Y has a half-life of only 63·4 hr., over 99% of any quantity of this element will decay in 19 days. When an organism takes into its body both ^{90}Sr and ^{90}Y the activity due to this yttrium may be neglected if sections of the tissues be set aside for 19 days before they are covered with photographic film. The resulting autoradiographs can be compared with those made from adjacent sections of the same animal which were covered with film a few hours after the tissues were fixed: the latter will show as the nuclear track in the emulsion of the film the position of β-particles from both the ^{90}Sr and ^{90}Y.

Uptake of ^{90}Sr by *Arion hortensis* as compared with that by an Opisthobranch

The land pulmonate stores in the tissues of its body calcium salts which may be used for the growth and repair of the shell and perhaps in buffering the gut (Manigault, 1939; Robertson, 1941). The lime cell of the digestive gland is the main storage place; also around blood vessels in both slug and snail are cells filled with calcium spherules, and in the mantle are the glands which secrete the shell (Prenant, 1924; Barr, 1928). These terrestrial molluscs obtain all their calcium from food: this mineral element, one of the most important in their metabolism, is preserved in the tissues in large quantities when it is available; in contrast, marine molluscs have a ready supply of calcium ions at hand in the surrounding medium and their reserves may be relatively low. In order to see whether the divalent ions of strontium follow a distribution which is similar to that of calcium in the body of a terrestrial, soft-bodied animal there is some advantage in using the slug or snail: they feed readily in captivity, and their calcium metabolism has already attracted the attention of many workers so that the distribution of the element within the tissues is well known.

Arion hortensis was fed with lettuce on which had been evaporated a solution of ^{90}Sr: the average consumption of isotope was of the order of 4 μc./g. of body weight, though a considerable amount did not enter the tissues, but was lost in the faeces. The slugs were given from one to four meals, each at night; they were fixed immediately after eating the contaminated food, or taken from this and put on uncontaminated leaves for a day or two before being fixed. Autoradiographs of the tissues of an individual which has been given only one meal with ^{90}Sr, and fixed immediately after it, show the isotope in both types of cell of the digestive gland—the digestive cell and the lime

cell, more in the former. It would appear that both take up the ions directly from the lumen of the gland and they are scattered in the cytoplasm. Such absorption by the digestive cell is well known; but no investigation has proved that the lime cell gets its calcium by this route, yet if strontium enters thus, as well as ^{32}P (Fretter, 1952), it is highly probable that calcium would do likewise.

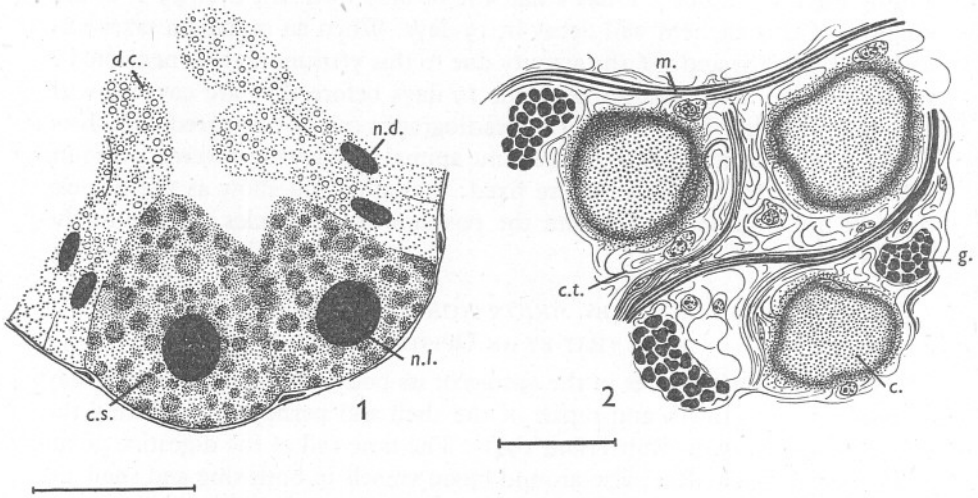

Fig. 1. *Arion hortensis*. Part of a transverse section through the digestive gland to show the distribution of ^{90}Sr. Stippling represents the superimposed autoradiograph. Fixed 3 days after commencing to feed on lettuce on which had been evaporated a solution of ^{90}Sr. *c.s.*, calcium spherule; *d.c.*, digestive cell; *n.d.*, nucleus of digestive cell; *n.l.*, nucleus of lime cell. Scale, 30 μ.

Fig. 2. *Acanthodoris pilosa*. Part of a transverse section through the periphery of the mantle to show the accumulation of ^{90}Sr. The specimen had been in activated, filtered sea water for 17 hr. and then in normal sea water for 12 hr. Stippling represents the superimposed autoradiograph. *c.*, calcium concretion; *c.t.*, connective tissue; *g.*, gland cell; *m.*, muscle fibre. Scale, 40 μ.

Slugs which have eaten larger quantities of the tracer over a period of 3 days show, in autoradiographs of their tissues, localization of strontium ions around the calcium spherules which fill the lime cells (Fig. 1, *c.s.*), and a general scattering in the rest of the cytoplasm of these cells; similar individuals, which have been kept on uncontaminated lettuce for several days before fixation, show that elsewhere in the digestive gland little of the isotope occurs for it is concentrated for storage in the one type of cell. Ions would appear to enter this cell from the haemocoel—into which the tracer passes from the digestive cell (*d.c.*)—as well as from the lumen of the gland; the two routes have already been described for the entry of ^{32}P (Fretter, 1952). The digestive gland offers the largest, yet not the only area of absorption from the gut: the intestinal

epithelium is also permeable to ions. The slug, which feeds at night, retains undigested matter in the intestine during the following day. On its slow course towards the anus this food loses ions which pass into the surrounding epithelium; they are not stored here, but are lost directly to the haemocoel. The anterior aorta traverses the haemocoel and is surrounded by a reserve layer of calcium to which passes some of the strontium: a slug which has been starved for a week after feeding on the isotope shows that the element is still retained here. The third type of calcium cell, that in the mantle, may contain the tracer within 12 hr. after its entry into the gut; these cells take the isotope directly from the blood.

The few experiments with *Arion* reveal that strontium, which is in the same group in the periodic table as calcium, though an unlikely constituent of the natural diet, is taken up by the gut and concentrated in the calcium stores. It is very probable, however, that there are quantitative differences between the uptake of the two elements by the storage tissues. Now in a marine animal, divalent cations may enter the tissues with the food, by way of the gut, or, as Bethe (1934) has shown for *Aplysia*, Ca^{2+} and Mg^{2+} may penetrate the integument from the surrounding water. Bethe's work was not extended to strontium which, present only in traces in sea water, is of much less importance. However, if a small specimen of *Aplysia punctata*, in which the buccal cavity is carefully ligatured without damage to the surrounding tissues, be placed in activated water for 8 hr., autoradiographs of the whole animal show the passage of strontium ions through the general surface of the body; the gill with its thin wall and active transport systems displays in its tissues the greatest activity.

A comparatively rapid accumulation of strontium from the surrounding water is seen in the nudibranch *Acanthodoris pilosa*. It is well known that dorids may have an unusually high amount of mineral matter which is concentrated in the mantle. An examination of the composition of the body tissues of *Archidoris britannica*, after removal of mucus and visceral mass, shows that calcium comprises 2% fresh weight, magnesium 0·93%, and strontium 0·08% (McCance and Masters, 1937); this proportion of strontium to calcium is higher than in sea water. In *Acanthodoris*, as in *Archidoris*, the mineral deposits scattered through the mantle give solidity to the thick covering of the body. McCance & Masters suggest that such nudibranchs, which in the course of evolution have lost the shell, never acquired the necessary excretory mechanism for dealing with ingested calcium and consequently accumulate it. If a specimen of *Acanthodoris* be placed in sea water which has been passed through a Berkefeld filter (KF grade) and activated with 90 μc. ^{90}Sr/l., autoradiographs of the tissues show that, after 10 hr., strontium ions which have passed into the body are most numerous in the mantle, gathering around the calcium concretions there; no such concentrations occur elsewhere (Fig. 2, *c*.). Some ions, though relatively few, are in the

lumen of the gut. This, as well as the outer surface of the body, may be the route by which they have entered. An individual which has been in the activated water for 36 hr., and then in normal sea water for a day, shows the strontium localized around the mineral concretions and in the cells which surround these (Fig. 4B). Some ions are leaving the body with waste matter from cells of the digestive gland. The radioactive strontium which has gathered in the mantle represents an exceedingly small amount, yet it indicates that in the nudibranch the mineral ions may be derived directly from the water, as well as by way of the food.

Uptake and Excretion of ^{90}Sr by *Mytilus edulis*

Marine lamellibranchs have attracted the attention of more scientific workers than any other group of molluscs, yet many problems of their metabolism are imperfectly understood: some of these concern the growth of the calcareous shell. This growth continues during periods of starvation and, since there is little storage of calcium in the tissues, points to the uptake of salts directly from the water. If uptake occurs it would appear to be to no slight degree: Chatin & Muntz (1895) calculated that 75 g. of shell formed by an oyster in 12 months correspond to about 27 g. calcium; this means an absorption of 0·52 g. per week. Orton (1935) puts the weekly absorption rate at about a third of this, and he states (1925) that the shells continue to grow in the absence of food. Later Galtsoff (1934), in studying the Virginia oyster, finds that from November to the end of April, when these animals are for the greater part of the time in a state of hibernation and have no food in the digestive tract, the weight of the soft tissues remains practically stationary whilst that of the shell increases at about the same rate as during the months of feeding. Such statements indicate that calcium from the water is taken up directly into the tissues of the bivalve: yet Robertson (1941) failed to detect direct uptake of calcium ions from sea water in certain common gastropods and lamellibranchs. It may be that the methods he employed were not sufficiently sensitive to detect minute quantities.

Fox & Coe (1943), in describing the biology of the Californian sea mussel (*Mytilus californianus*), state that an individual measuring 100 mm. in length accumulates in its tissues and shell 17 g. calcium in a year; the calcium is not available in such quantities in the food, and must, they say, be obtained from the water in particulate or soluble form. If the tissues are permeable to ions such a quantity of calcium can be readily obtained: Fox, Sverdrup & Cunningham (1937) have shown that a medium-sized mussel may pass water through its gill chambers at an average rate of 22,000 l. a year, provided filtration continues incessantly, and this volume of water would contain about 9·2 kg. of calcium as salts in solution or suspension; an individual need utilize only a very small quantity of this to obtain the required 17 g. Yet if the calcium in artificial sea water be cut down to one-eighth its normal value the organic

matter of the newly formed shell of *Pedalion* is deposited in the usual way (Bevelander & Benzer, 1948), but there is no calcification—the animals in this experiment may be influenced by factors other than the low calcium content of the artificial medium.

Korringa (1952) suggests that the oyster collects calcium ions from the sea in much the same way as it extracts and accumulates other positive polyvalent ions such as Fe, Cu, Zn, Fe, Hg and Mn, which are present in sea water only in small amounts: ions, if adherent to the mucous feeding sheets would be automatically collected and ingested. The use of autoradiographs presents an opportunity of finding whether this occurs, and may indicate which tissues accumulate specific ions. From results obtained by this method Bevelander (1952) concludes that both fresh-water and marine molluscs take up labelled calcium and phosphate ions. The experiments on which these conclusions are based concern only lamellibranchs: mantles and shells were removed and autographs made only of these parts; there was, apparently, no attempt to trace the course of the ions or their localization in any other part of the body.

Uptake

Small *Mytilus* measuring 1·0–1·5 cm. in length were chosen for the present study so that autoradiographs could be made of the entire body; most results were then verified on larger specimens. The activity of the sea water in which the experimental animals were placed varied from 30 to 100 μc./l.; sometimes this water contained a culture of dinoflagellates, at other times it had been passed through a Berkefeld filter. Doses up to 450 μc./l. did not appear to damage the tissues. Much of the yttrium and some strontium would be adsorbed on the vessel in which the animal was kept, and on its shell. The higher doses give an insight into the way in which the mussel may deal successfully with an unusual influx of ions into the body.

When dinoflagellates are present in the activated water a larger amount of tracer enters the body of the mussel, for the flagellates concentrate the ions to some extent and are readily collected by the feeding currents. It is seen from autoradiographs that after *Mytilus* has been in water with these flagellates for $2\frac{1}{2}$ hr. the stomach displays the area of greatest radioactivity, and the tracer has passed to the digestive gland where strontium and yttrium ions are most numerous in the distal part of the cytoplasm of the digestive cells, and are scattered proximally. Some activated food has by this time entered the intestine, and from it ions of both ^{90}Sr and ^{90}Y penetrate the epithelium of the intestine and enter the haemocoel. Absorption of ferric saccharate by the intestine of a marine mollusc has been described by Gabe & Prenant (1949) for chitons, though these authors do not mention the fate of the iron which is taken up in this way. Some preliminary work with autoradiographs of *Lepidochitona cinereus* and *Patella vulgata* shows that the intestinal epithelium in both of these molluscs is permeable to strontium ions which pass by this

route to the haemocoel. The long intestine in the herbivore is known to be concerned with the elaboration of faeces (Graham, 1932) and its permeability to ions may be of some importance; the food is slow to pass along it and the faeces are of considerable bulk. Yonge (1926*a*), working with *Ostrea edulis*, finds no evidence of absorption in the epithelium of the gut. He is not, however, concerned with passage of ions. It is easy to imagine that an epithelium may act as an area of ion absorption or exchange, yet be impermeable to the droplets of oil, blood corpuscles, or small diatoms which Yonge employed and which he finds are taken up by cells of the digestive gland and by phagocytes only. In *Mytilus* some strontium ions, though fewer, pass through the wall of the stomach.

In experiments with filter-feeders it is difficult to prevent the entry of particulate matter into the gut. Even if the shell of *Mytilus* be thoroughly cleaned and the animal kept in Berkefeld-filtered water (KN grade of filter) 3 days to clear the larger particles of detritus from the mantle cavity before the experiment, the results may occasionally show such particles in the gut. These preparations were made for the experiments which are cited below. MacGinitie (1945) has shown that in *Urechis* (Echiuroidea) particles between 36 and 90 Å. in diameter may be caught by the mucous feeding sheets and with them enter the alimentary canal; such food may form an important part of the diet of a microphagous feeder. In order to prevent entrapped particles from entering the mouth of *Mytilus* attempts have been made to block the oral opening without injury to any part of the body, but these did not meet with complete success.

If small *Mytilus* be placed in filtered sea water activated with 100 μc. ^{90}Sr/l. autoradiographs of the sectioned tissues show that after 2 hr. strontium ions are present in the lumen of the gut, in the digestive gland, and in the tissues of the gill filaments. Their occurrence elsewhere in the body will be neglected for the present. In some specimens ions may be adherent to the outer surfaces of the gill filaments and labial palps, also to secretion which may be in contact with these surfaces. The amount of tracer which has entered the body of an individual is greater than could have been admitted into the alimentary canal with occasional particles in the filtered sea water: tracer ions have been taken up directly into the tissues. Their entry may be by one of two routes, or by both of these. The extremely large surface presented by the single pair of ctenidia of a lamellibranch may offer an area for ionic exchange or absorption, even though, as Yonge (1928) has shown for *Ostrea*, the surface is impermeable to molecules of glucose. Ronkin (1950), using excised fragments of the gills of *Mytilus edulis*, traced a slow penetration of ^{32}P into the tissues from the artificial sea water with which they were irrigated: chemical and radioactive assay revealed that only 0·06% of the intracellular phosphate was exchanged after the tissues had been exposed for 140 min. Such an experiment cannot refer to uptake by the outer surface of the epithelium alone, since the cut ends

of the filaments were exposed to the external medium and this might penetrate through the open blood spaces. However, Ronkin assumes that ionic exchange takes place at the surface of the ctenidium, and most of this, he suggests, is for use in that organ. The filaments receive their blood from the kidney and other capillaries: their blood pressure is low and the flow through them too slow to serve as an efficient transport mechanism from their tissues to other parts. It is apparent from autoradiographs that strontium ions enter the body by this route. If a medium-sized *Mytilus* has the shell wedged open slightly to give free access to the mantle cavity, and is placed so that its posterior half is in activated sea water, after 40 min. some strontium ions are within the ciliated cells of the gill filaments. Since no activity can be traced within the gut, and since in so short a time it is unlikely that ions, which might have entered by this route, would have circulated in the blood stream and be picked out almost specifically by these cells, it is assumed that the strontium has made direct entry to the ciliated epithelium of the gills. However, such entry of ions into the body would not appear to be the major one. The primary function of the gills is feeding, and ions which adhere to the mucus secreted by them may automatically find entry to the mouth. Korringa (1952) thinks it possible that the electrical properties of both food particle and feeding sheet may determine whether or not particles are readily caught by a bivalve: the oyster takes up into its body the positive polyvalent ions which have been previously mentioned, though positive monovalent ions like Na^+ and K^+, and negatively changed ions, are not easily caught. If this be so then strontium ions should be readily collected. No *Mytilus* which has been sectioned shows the mucous feeding sheets; these, if present, were destroyed in the processing for autoradiographs. In occasional individuals secretion on the ctenidial surface and on the labial palps is present, and to this some ions adhere. *Mytilus* which have been left for varying periods—a few hours to 3 days—in filtered sea water activated with ^{90}Sr all suggest that the gut is the more important area for ingress of ions to the tissues; presumably they have been directed there by the feeding currents. Their concentration is greatest in the digestive gland.

If strontium enters so readily the tissues of the gills, and, more especially, the gut, there is no reason for believing that the divalent ions of calcium would not follow a similar course and so provide for the continuous growth of the shell even in starved individuals.

The digestive gland of lamellibranchs consists of one type of cell (Yonge, 1926*b*) which is concerned with ingestion and digestion. Strontium and yttrium ions which pass into the gland in *Mytilus* are not accumulated for storage in these cells: they enter the haemocoel and are circulated to all tissues of the body. Bevelander (1952) in his discussion on calcification in molluscs states that 'calcium ions present in the water are ingested by the organism and are localized in several regions'. Of these regions he mentions only the

mantle saying that the localization there is essentially the same in both freshwater and marine molluscs and he figures an autoradiograph of *Anodonta grandis* with tracer calcium concentrated in the periphery, slightly below the epithelium and in the epithelial cells themselves. In a similar position Trueman (1942) finds scattered calcium stores in *Tellina tenuis*. If strontium follows the path of calcium one might expect to find in experimental animals an accumulation of ions in these same parts which differentially select material for the production of the shell. Small *Mytilus* which have been in activated sea water up to 5 days and then in normal sea water for periods ranging from a few hours to 3 days show only a scattering of strontium in these mantle tissues and no pronounced localization. It may be that the experiments were carried on for too short a time or, rather, that the more abundant unlabelled calcium ions are preferentially selected. Actually in the mantle of *Mytilus* there is no heavy storage of calcium which might accumulate the divalent cations as a reserve comparable to that in the liver of a slug. If crystal filaments of shell be accidentally autographed with the soft tissues, however, a layer of adsorbed strontium ions is seen to encircle each. Normally strontium, if present in the lamellibranch shell, is only in very small amounts: Trueman (1942) has shown that in *Tellina tenuis*, *T. baltica* and *Donax vittatus* the shell contains traces of strontium which he suggests may contribute towards the formation of aragonite.

Excretion

Perhaps the most surprising results from the present experiments concern the rapidity with which the ions of both strontium and yttrium are accumulated for excretion, bearing in mind that the strontium, as displayed in autoradiographs, is an indication of the events of not more than 0·07% of the total quantity present in the sea water for concentrations of 100 μc./l. of water. Presumably a much greater percentage of unlabelled ions are following a similar course through the tissues of the mussel.

The excretory system of lamellibranchs consists of two separate groups of organs, the kidneys and the pericardial glands; to these may be added the digestive gland which, though with other major functions to perform, liberates to the intestine waste from its own metabolic activities. Takatsuki (1934) found that in *Ostrea edulis* these organs work in conjunction with amoebocytes which free the body of foreign or indigestible particles: they ingest these and remove them by way of the excretory organ, pericardium, surface of the auricle, rectum and mantle cavity; no such particles are taken up directly by cells of the excretory tubules. *Mytilus* which have been in activated water for 10 hr. show that cells of the pericardial gland are accumulating the tracer elements (Fig. 3A). These glands (*p.g.*) surround the auricles (*a.*) and so come into contact with the blood from which the ions are extracted; there is no indication that they have entered the cells of the gland exclusively, or even to any

appreciable extent, by means of amoebocytes transporting them there as described for the course of waste particulate matter in *Ostrea* (Takatsuki, 1934). As more tracer enters the body of the mussel, with longer periods of

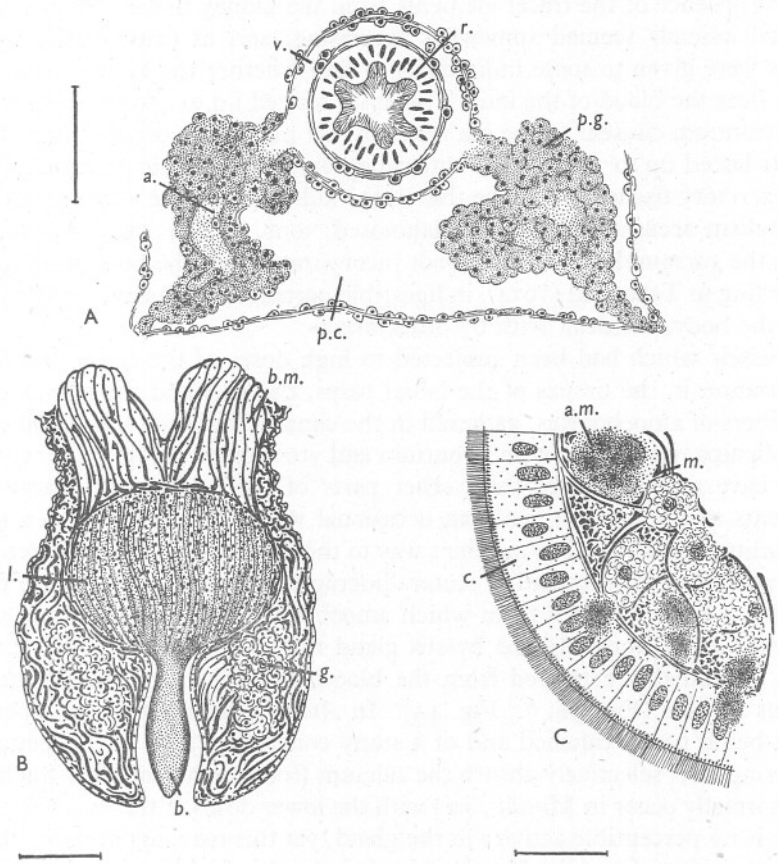

Fig. 3. *Mytilus edulis*. A, transverse section through the heart and pericardial glands. Stippling represents the superimposed autoradiograph. The mussel had been in activated sea water for 10 hr. Scale, 250 μ. B, transverse section through the foot, after the mussel had been subjected to a high dose of ^{90}Sr. Scale, 500 μ. C, part of a transverse section through a labial palp, after a high dose of ^{90}Sr. Scale, 25 μ. Stippling represents the superimposed autoradiograph. *a*, auricle; *a.m.*, amoebocyte; *b.*, byssus thread; *b.m.*, byssus retractor muscle; *c.*, ciliated epithelium; *g.*, byssus gland in foot; *l.*, glandular lamellae dividing byssogenous cavity; *m.*, muscle fibres; *p.c.*, pericardial cavity; *p.g.*, pericardial gland; *r.*, rectum; *v.*, ventricle.

exposure to the activated water, so a heavier accumulation of ^{90}Sr and ^{90}Y is found in the pericardial gland. Cuénot (1899) states that the phagocytic cells of the glands which capture particles may eventually transport them from the

gland: they deposit the waste in connective tissue or carry it from the body by way of the gills or palps. Alternatively, the phagocytic cells may discharge their contents directly into the pericardial cavity (Takatsuki, 1934), which is in communication with the lumen of the kidney by the renopericardial duct.

The absence of the tracer elements from the kidney tissue of these experimental animals seemed somewhat surprising, and in consequence heavier doses were given to some individuals to find whether the kidney cells might then clear the blood of the ions. The doses ranged up to 450 μc./l., increasing the strontium content of the sea water by not more than 0·315% and experiments lasted up to 3 days. Yet autoradiographs revealed no accumulation by the excretory tissue. It may be that in the kidneys only the waste products of katabolism accumulate and are elaborated: ions, as ^{90}Sr and ^{90}Y, taken up from the surrounding water and not incorporated into the body tissues, and, according to Takatsuki (1934), indigestible particles which have been injected into the body, are dealt with by other means.

Mussels which had been subjected to high doses of the tracer showed its localization in the tissues of the labial palps, the gills and the byssus gland. Numbers of amoebocytes, gathered in the connective tissue and blood spaces of the palps, were laden with strontium and yttrium ions (Fig. 3C, *a.m.*): they may have migrated here from other parts of the body to discharge their contents to the exterior; only an occasional wandering cell was seen in the epithelium—if any had found their way to the surface they had been removed on fixation, or subsequently. Autoradiographs gave a similar picture of the blood spaces of the gills from which amoebocytes pass to the mantle cavity carrying excess ions. In the byssus gland the tracers were in the secreting cells, presumably extracted from the blood, and they were passed into the byssus threads (Fig. 3B, *b.*; Fig. 4A). In *Anomia* the byssus is of a peculiar form being partly calcified and of a stony consistency, for in this genus the secreting cells selectively absorb the calcium from the haemocoel. Such does not normally occur in *Mytilus*, and with the lower doses of the tracer elements there is no perceptible activity in the gland, yet this may, apparently, liberate divalent cations from the blood when their level is high.

Mention must still be made of the excretory function of the digestive gland. The tubules comprise only one type of cell (Yonge, 1926*b*) which is active in the uptake of ions from the gut. After a period of feeding yellow concretions may be found to accumulate in a distal vacuole of the cytoplasm—presumably the waste products of cellular metabolism, including digestion. Concretions are expelled to the lumen of the gland and so reach the intestine: since a large number of digestive cells may excrete at any one time the volume of this faecal matter can be quite considerable. Mussels which have been in activated, filtered sea water from 3 to 5 days and then in normal sea water for a corresponding length of time, show strontium and yttrium ions leaving the body with waste from the gland, and the tracers can be found leaving the cells with

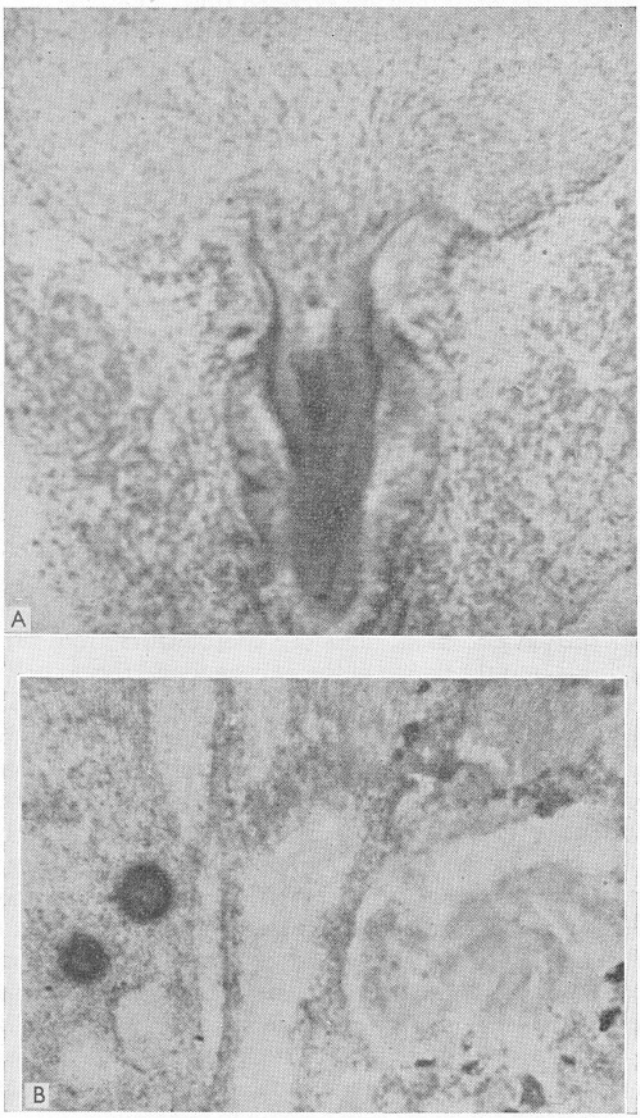

Fig. 4. A, *Mytilus edulis* subjected to a high dose of ^{90}Sr. Photomicrograph of part of a transverse section through the foot, with superimposed autoradiograph, to show ^{90}Sr and ^{90}Y in the byssus thread. B, *Acanthodoris pilosa*, photomicrograph of part of a transverse section through the mantle, kidney and hermaphrodite gland, with superimposed autoradiograph, to show ^{90}Sr localized around two mineral concretions in the mantle.

the yellow concretions. Some ions taken from the gut by the digestive cells may never leave them until they are excreted thus. This would not appear to happen to all, for there is evidence that ions which have circulated in the blood may eventually be taken back by the digestive cells to be freed from the body. Such evidence comes from animals which after taking up the tracer from filtered water are placed in normal sea water for a number of days: the digestive gland may show an accumulation of ions which is just as heavy after 5 days in normal sea water as after one, although during those 5 days excreta from the gland, carrying the tracer with it, have passed to the intestine.

Wandering Phagocytic Cells in *Calyptraea chinensis* and *Platynereis dumerili*

The importance of phagocytes in the metabolic activity of lamellibranchs has long been recognized (Cuénot, 1899; Yonge, 1926*a*, *b*). Occurring in large numbers they provide a source of digestive and excretory activity. In their cytoplasm are elaborated digestive enzymes to deal with such ingested particles as diatoms, and even blood corpuscles; their excretory function, at least in *Mytilus*, is extended to the uptake of excess ions, for which they may take the role of an emergency system. Less recognition has been given to the phagocytes of gastropods. Millott (1937) suggests that in nudibranchs they are of significance in excretion, ingesting effete matter from the haemocoel and discharging it into the lumen of the gut; he found this to be their activity with particles of iron saccharate. Prosobranchs may have phagocytes which are similarly excretory in function. If specimens of *Calyptraea* be placed in filtered sea water activated with ^{90}Sr to the extent of 100μc./l., ions pass into the body, and, circulated in the blood stream, they may be carried to all tissues. Autoradiographs of individuals which have been in activated water for 2 days show concentrations of strontium and yttrium in phagocytic cells. These may occur in various parts of the body: the tissues of the foot, the bases of the gill filaments, the wall of the pericardium and of the kidney. It is doubtful whether these positions can be connected with any nutritive function of the wandering cells. Whatever their action may be it is associated with an increase of strontium in the external medium of 0·70%: in the body tissues the increase could therefore be only slight. This suggests that the phagocytes are extremely sensitive to changes in their environment. Perhaps one of their important functions is in helping to maintain an ionic steady state within the body. The localization of ^{90}Sr is also marked in the hypobranchial gland. It elaborates large quantities of secretion, and in taking up constituents from the blood presumably abstracts the divalent cations of strontium, which are then freed from the body with its secretion.

Wandering amoebocytes occur in polychaete worms. Romieu (1923), referring to them as leucocytes, says that they may collect excretory matter

from the body and transport it to the epidermis, where they may be seen in *Dodecaceria concharum*. Similar phagocytosis by leucocytes occurs in nereids and *Aphrodite aculeata* (Fordham, 1925); the cells are particularly large and active in *Perinereis cultrifera* (Romieu, 1923). In polychaetes it is recognized that one type of leucocyte may deal with waste, another with food stuffs. The former, which phagocytose and eliminate noxious material, are called lymphoidocytes; the latter, which transport and release food into the blood and tissues, are trephocytes. Liebman (1946) describes and figures the two in *Amphitrite crinata*. Since in certain molluscs amoebocytes are active in the transport of ions it was thought that the investigation of a polychaete might show that in this class also they can be similarly employed. Autoradiographs of sections of *Amphitrite gracilis* and *Platynereis dumerili* which had been

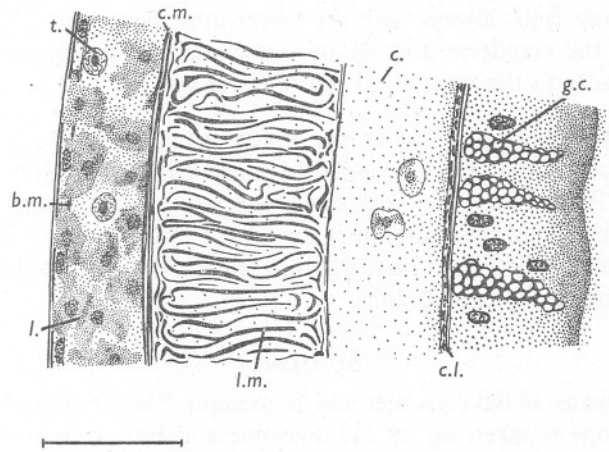

Fig. 5. *Platynereis dumerili*. Part of a transverse section. Stippling represents the superimposed autoradiograph. The specimen had been in sea water activated with ^{90}Sr for 3 days, and had taken activated food into its gut. *b.m.*, basement membrane of ectoderm; *c.*, coelom; *c.l.*, circular and longitudinal muscles of gut; *c.m.*, circular muscle of body wall; *g.c.*, gland cell in epithelium of gut; *l.*, lymphoidocyte; *l.m.*, longitudinal muscles of body-wall; *t.*, trephocyte. Scale, 250 μ.

subjected to the dose of 100 μc. ^{90}Sr/l. of sea water, showed no use of amoebocytes in dealing with tracer ions which have entered the tissues. *Platynereis* were then kept for 3 days with 3 times as much tracer in the water, some in filtered sea water, and others amongst small quantities of sand and detritus from the coralline pool in which they had been collected. Autoradiographs of those individuals revealed wandering cells laden with both strontium and yttrium ions (Fig. 5, *l.*). They were found most commonly beneath the ectoderm (*b.m.*) of the body wall, a position which suggests that they are lymphoidocytes ridding the body of the tracer elements. The greatest amount of radioactivity was found in a worm which had ingested a fragment of weed

together with some detritus; from these contents ions passed through the wall of the alimentary canal (*g.c.*) to the coelom (*c.*). The gut would appear not to be the only path by which ions enter the body, although they find entry here even in the absence of food: individuals kept in radioactive sea water for half an hour show little tracer in the alimentary canal and more in the integument, suggesting an absorption here. Lymphoidocytes with some ^{90}Sr and ^{90}Y are occasionally in the coelom: always those with the highest concentration are clustered between the basement membrane of the ectoderm and the underlying fibres of circular muscle (*c.m.*). Amongst them occurs an apparently less frequent type of cell with little or no tracer in its cytoplasm. This is presumably the trephocyte (*t.*) which is concerned with the transport of nutritive material. The subepithelial clusters of lymphoidocytes may become very pronounced if a worm be subjected to high doses of the tracer for several days. Some of these cells, always with the tracer ions, have been found between the cells of the ectoderm and on its outer surface, behaving in a fashion which is similar to the wandering cells of *Mytilus*.

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Summary

If *Arion hortensis* be fed on a diet which contains ^{90}Sr, autoradiographs show that the isotope is taken up by the digestive and lime cells of the digestive gland. From the former it passes to the haemocoel; in the lime cells it is concentrated around the calcium spherules. Some of the tracer enters the body through the wall of the intestine. Calcium stores which surround blood vessels and calcium cells in the mantle also concentrate the tracer.

In *Aplysia punctata* ^{90}Sr from the surrounding water passes through the surface of the body, and especially the gill; in *Acanthodoris pilosa* ions which enter the tissues from the sea water accumulate around the numerous calcium concretions in the mantle. These marine molluscs obtain cations directly from the water as well as by way of the food.

There is a slow uptake of strontium ions by the ctenidia of *Mytilus edulis*, though, even in filtered sea water, the gut is the more important area for their ingress to the body. It is possible that they enter with the mucous feeding-sheets. They pass readily into the cells of the digestive gland. Some of the isotope taken in with the food is absorbed by the wall of the intestine; this also occurs in *Patella vulgata*, in which the intestine provides a much larger area, and in *Lepidochitona cinereus*.

Mytilus placed in filtered sea water which is activated with ^{90}Sr, so increasing the strontium content by 0·07%, show the tracer localized for excretion within 10 hr. Ions are aggregated in the pericardial glands, not in the kidney. If the strontium content of the water in which *Mytilus* are kept be increased by the tracer up to 0·315%, within 3 days amoebocytes with the element, and with its daughter product yttrium, are gathered in the connective tissue of the labial palps, and in blood spaces of the gills from which they pass to the mantle cavity. The tracers also leave the body with secretion from the byssus gland, but are not accumulated by the cells of the kidney. There is no storage of ^{90}Sr by the digestive gland which excretes the ions into the gut, nor are there high concentrations retained by tissues of the mantle.

Amoebocytes of *Calyptraea chinensis* accumulate the tracer element which enters the tissues from filtered sea water. One of their important functions may be in helping to maintain an ionic steady state within the body.

Two types of wandering cells occur in *Platynereis dumerili*: one of these, the lymphoidocyte concentrates the isotope in its cytoplasm and transports it to the outer surface of the body.

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