Vol. 52: 217–225, 1989 | MARINE ECOLOGY PROGRESS SERIES
Mar. Ecol. Prog. Ser. | Published March 22

Histo-autoradiographic localisation of americium (^{241}Am) in tissues of European lobster *Homarus gammarus* and edible crab *Cancer pagurus* after uptake from labelled sea water

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ABSTRACT: Uptake from sea water and cellular localisation of americium were studied by autoradiography in the European lobster *Homarus gammarus* and the edible crab *Cancer pagurus*. Marked uptake was noted in the gills; americium was adsorbed by the mucus between the gill lamellae in the lobster and by the chitinous surface of the gills in the crab. Americium uptake in the hepatopancreas was intracellular, and alpha traces were noted in all cell types of the hepatic tubules, secretory cells, fibrillar cells, and particularly the resorptive cells. Americium was also localised in the cells of the labyrinth of the green gland, indicating the role of this gland in elimination of americium.

INTRODUCTION

Experimental data (Ward 1966, Fowler et al. 1975, Guary & Fowler 1977, 1978, Fowler et al. 1986) and in situ (Guary et al. 1976, Pentreath et al. 1985) on the transfer and distribution of transuranic elements in decapod crustaceans show that these elements are strongly adsorbed by external tissues (exoskeleton and gills) in direct contact with sea water. Penetration into internal tissues is minor, and occurs mostly in the hepatopancreas. The tissue localisation and penetration mechanisms of transuranic elements in decapod crustaceans are unknown. It is unclear if these elements are adsorbed by cellular epithelia of the digestive gland or whether localisation is intracellular. If the latter, it is not known whether the elements are distributed uniformly in the hepatopancreatic cells or whether accumulation is greater in certain cells. These uncertainties complicate evaluation of the effects of internal radiation due to uptake of transuranic elements.

Tissue localisation of transuranic elements by environmental measurements is complicated by inadequate labelling of organisms due to low concentrations of transuranic elements of the order of μBq l^{-1} from atmospheric fallout (Ballestra 1980), or mBq l^{-1} when industrial waste is discharged into the sea (Hetherington et al. 1975, 1976, Germain & Miramand 1984, Pentreath et al. 1984). We studied americium (^{241}Am) uptake in the macruran decapod *Homarus gammarus* (Eupopean lobster), and complementary information was provided by experiments with the brachyuran decapod *Cancer pagurus* (edible crab) which has a slightly different physiology.

Histo-autoradiography was used to localise americium in the tissues. This method does not result in modification of the histological structure and can be used to define the tissue localisation of the transuranic elements, by virtue of their alpha emission. This approach seemed of great interest to us since at present only a few studies have been carried out on the cellular localisation of transuranic elements in marine organisms (Miramand & Guary 1981, Leonard & Pentreath 1981, Galey et al. 1983, 1986, Miramand & Germain 1985).

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0171-8630/89/0052/0217/$ 03.00

MATERIALS AND METHODS

Three lobsters, each weighing about 200 g, and 3 crabs, each weighing about 100 g (all at stage C4), were individually labelled by uptake of americium from sea water over a period of 14 d. ^{241}Am (oxidation state III, $T_{1/2}$ 434 yr) in 1 N HNO_3 was prepared by the Commissariat à l'Energie Atomique (France). The labelling technique has been described previously (Miramand et al. 1982). The crustaceans were individually placed in tanks containing 15 l of filtered sea water (0.45 µm) (T = 14 ± 1 °C), and ^{241}Am (ca 14.8 Bq ml^{-1}, or 400 pCi ml^{-1}) was then added. The sea water was replaced every 48 h by water of the same quality containing the same radioisotope concentration. After labelling the specimens were dissected, and the radioactivity of tissues was measured. ^{241}Am X-ray emission (60 keV) was measured with 25 % efficiency with a gamma spectrometer coupled to a NaI (T1) well crystal. The gills, green glands and about 0.5 g of hepatopancreas were removed and placed in Bouin's fixative fluid. The tissues were prepared for histo-autoradiography according to the method described by Miramand & Guary (1981). After fixation, the tissues were dehydrated and impregnated with paraffin wax. Sections 5 µm thick were placed on glass slides, dried, and dewaxed. Autoradiographs were prepared with Ilford K2 nuclear emulsion (diluted 2-fold with 1 % glycerin solution) at 50 °C using the dipping technique (Rogers 1973). The slides were then dried, placed in hermetically sealed boxes and stored in the dark at 4 °C. Each week, autoradiographs were developed, fixed, rinsed and coloured with Masson trichrome. The slides were dehydrated and mounted for microscopic examination.

Mucopolysaccharides were tested for by Mowri's method (staining with Alcian blue) (Chevreau et al. 1977).

RESULTS

After labelling for 14 d, the lobsters were dissected and the different tissues radioanalysed. The concentration factors, CF (cpm g^{-1} wet wt/cpm ml^{-1}) and the relative distribution of americium in the tissues are given in Table 1. Marked americium uptake was noted in the gills (CF ≈ 130). These tissues contained 6 % of the total whole body americium content and nearly 63 % of the americium in the flesh. Fig. 1 shows a histological section of a gill labelled with americium. The black colouring between the gill lamellae is due to extensive overlapping of alpha traces generated by americium.

The staining of sections with Alcian blue demonstrates the superimposition of alpha traces and the blue-stained acid polysaccharides. Numerous alpha traces are also visible in the gill lamellae, but few are seen in the gill axes.

Americium uptake in crab gills was greater (CF ≈ 700), and considerable adsorption was noted in the external covering of the gill lamellae (Fig. 2).

Americium uptake in the hepatopancreas of the lobster was 10-fold less than in the gills; the concentration factor was ca 12 after 14 d of labelling. The digestive gland contained 0.7 % of the total body burden of americium and 8 % of the americium contained in the flesh. Fig. 3 shows a low-magnification micrograph of a section of the hepatopancreas. The arrangement of the hepatic tubules and the various cells that comprise them can be seen (Barker & Gibson 1977). In particular, it is possible to identify: the secretory cells (B cells) which contain a single large vacuole, the more numerous resorptive cells (R cells) which contain abundant lipid reserves and are clear, and the fibrillar cells (F cells) which are more heavily coloured with Masson Trichrome. The small embryonic cells (E cells) restricted to the terminal blind-ending regions of each

Table 1. *Homarus gammarus*. Concentration factor (CF) and percentage content of ^{241}Am in various tissues of the lobster after 14 d exposure in contaminated sea water (n = 3 individuals)

Tissue/organ	CF	% Total ^{241}Am content	% Total wet wt	% Soft part ^{241}Am content	Soft part % wet wt
Exoskeleton	90 ± 18	84 ± 18	50 ± 4		
Gill	130 ± 16	5.7 ± 1.0	2.5 ± 0.5	62.8 ± 8.0	8.6 ± 1.7
Hepatopancreas	12 ± 5	0.7 ± 0.3	3.5 ± 0.2	8.1 ± 3.5	12.4 ± 1.0
Digestive tract	7 ± 3	0.2 ± 0.1	1.8 ± 1.0	2.3 ± 1.2	6.5 ± 3.6
Green gland	4 ± 2	0.02 ± 0.01	0.1 ± 0.05	0.2 ± 0.1	0.3 ± 0.1
Muscle	2 ± 1	0.9 ± 0.5	30 ± 1	9.3 ± 4.7	68.2 ± 2.3
Epidermis	79 ± 20	1.6 ± 0.5	1.1 ± 0.2	17.4 ± 4.4	4.0 ± 0.7
Remaining tissues		6.7 ± 1.5	11 ± 2		

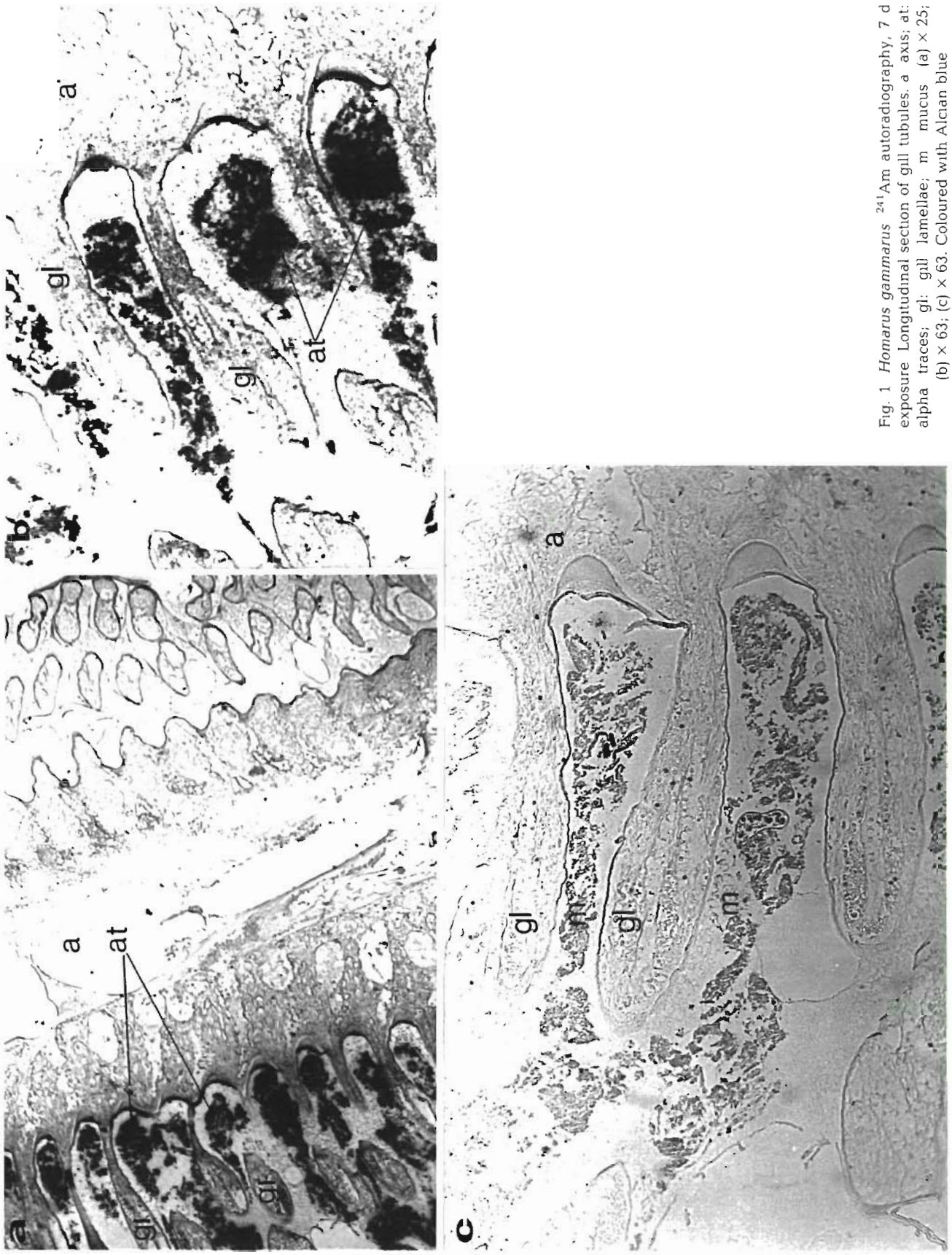

Fig. 1 *Homarus gammarus* ^{241}Am autoradiography, 7 d exposure Longitudinal section of gill tubules. a axis; at: alpha traces; gl: gill lamellae; m mucus (a) × 25; (b) × 63; (c) × 63. Coloured with Alcian blue

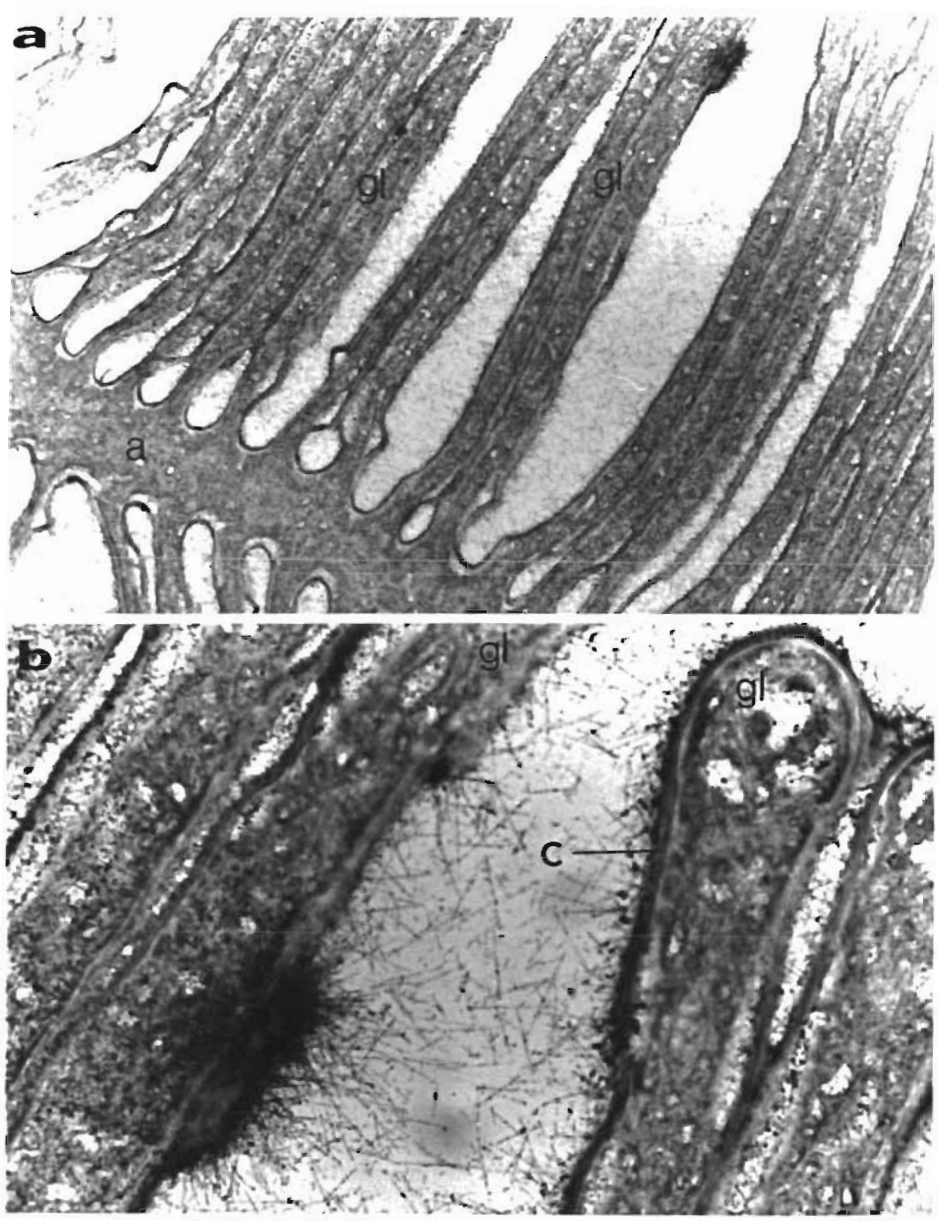

Fig. 2. *Cancer pagurus*. ^{241}Am autoradiography; 30 d exposure. Longitudinal section of gill tubules. a: axis; c: cuticle; gl: gill lamellae. (a) × 100; (b) × 400

tubule are not visible. At higher magnification, alpha traces are visible inside the hepatic tubules (Fig. 3), and the cellular localisation of americium is clear. Alpha traces were noted in all cell types, in particular in the resorptive cells. Alpha traces were also visible in the lumina of the tubules, and were therefore associated with the mucus present. Uptake of americium in the hepatopancreas (CF ≈ 7) of the crab was about 100-fold less than in the gills. The localisation of americium was identical to that seen in the lobster (Fig. 4).

The green gland (= antenna gland) is the excretory gland in decapod crustaceans. After 14 d of americium uptake in lobsters, the green gland was weakly labelled (CF ≈ 4) (Table 1).

Histological sections were taken from the glandular part of this organ, the labyrinth, a spongy mass with a large surface area in contact with the haemolymph. An enlargement shows the distribution of the alpha traces inside the labyrinth cells. The interior canals were virtually unlabelled (Fig. 5).

DISCUSSION

When lobsters and crabs were labelled by uptake of americium from sea water, the radionuclide accumulated considerably on the outer surfaces in contact with the immediate environment; the exoskeleton and the gills bound nearly 90 % of the total body burden of americium. The results for americium were similar to

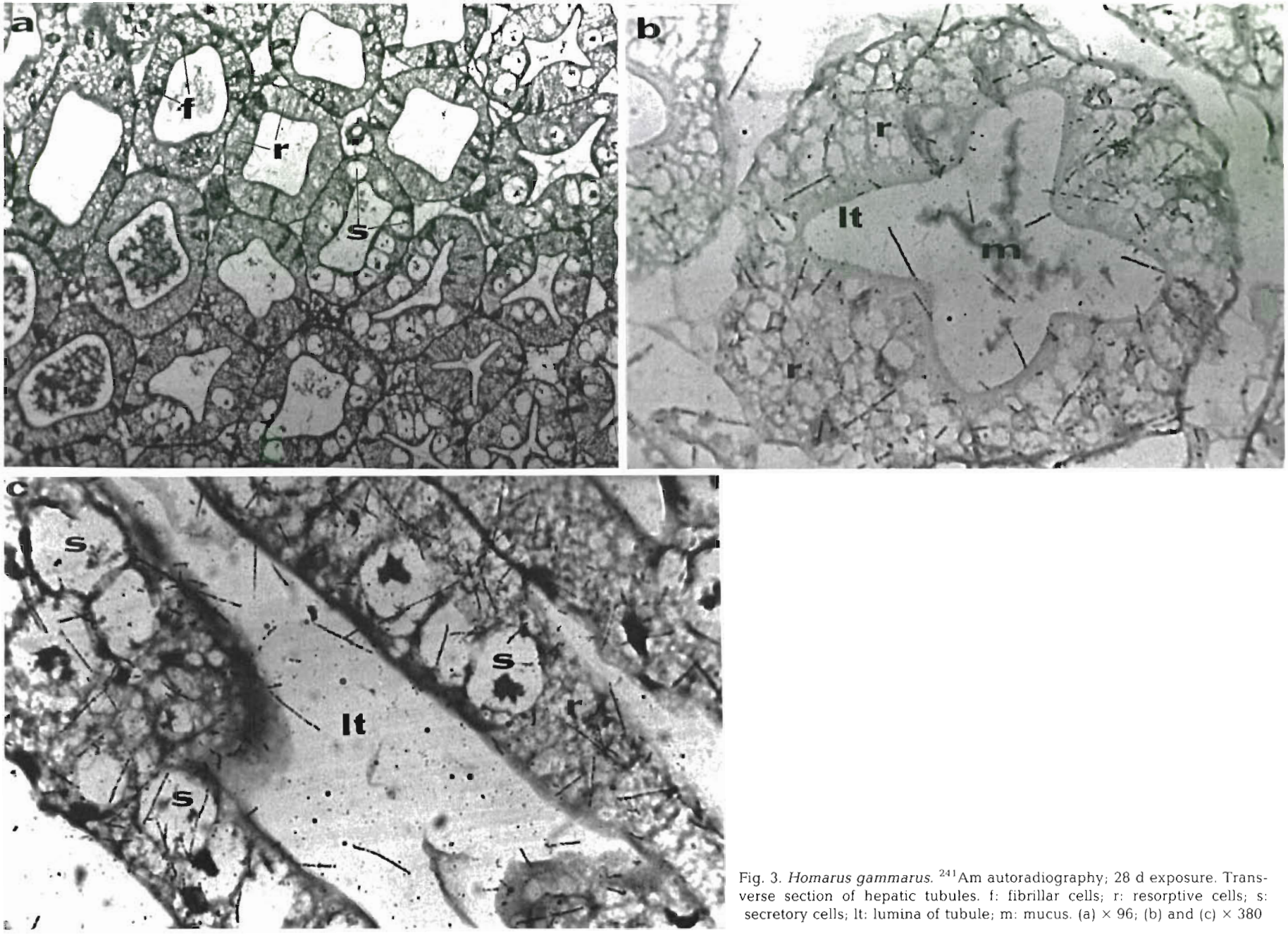

Fig. 3. *Homarus gammarus.* ^{241}Am autoradiography; 28 d exposure. Transverse section of hepatic tubules. f: fibrillar cells; r: resorptive cells; s: secretory cells; lt: lumina of tubule; m: mucus. (a) × 96; (b) and (c) × 380

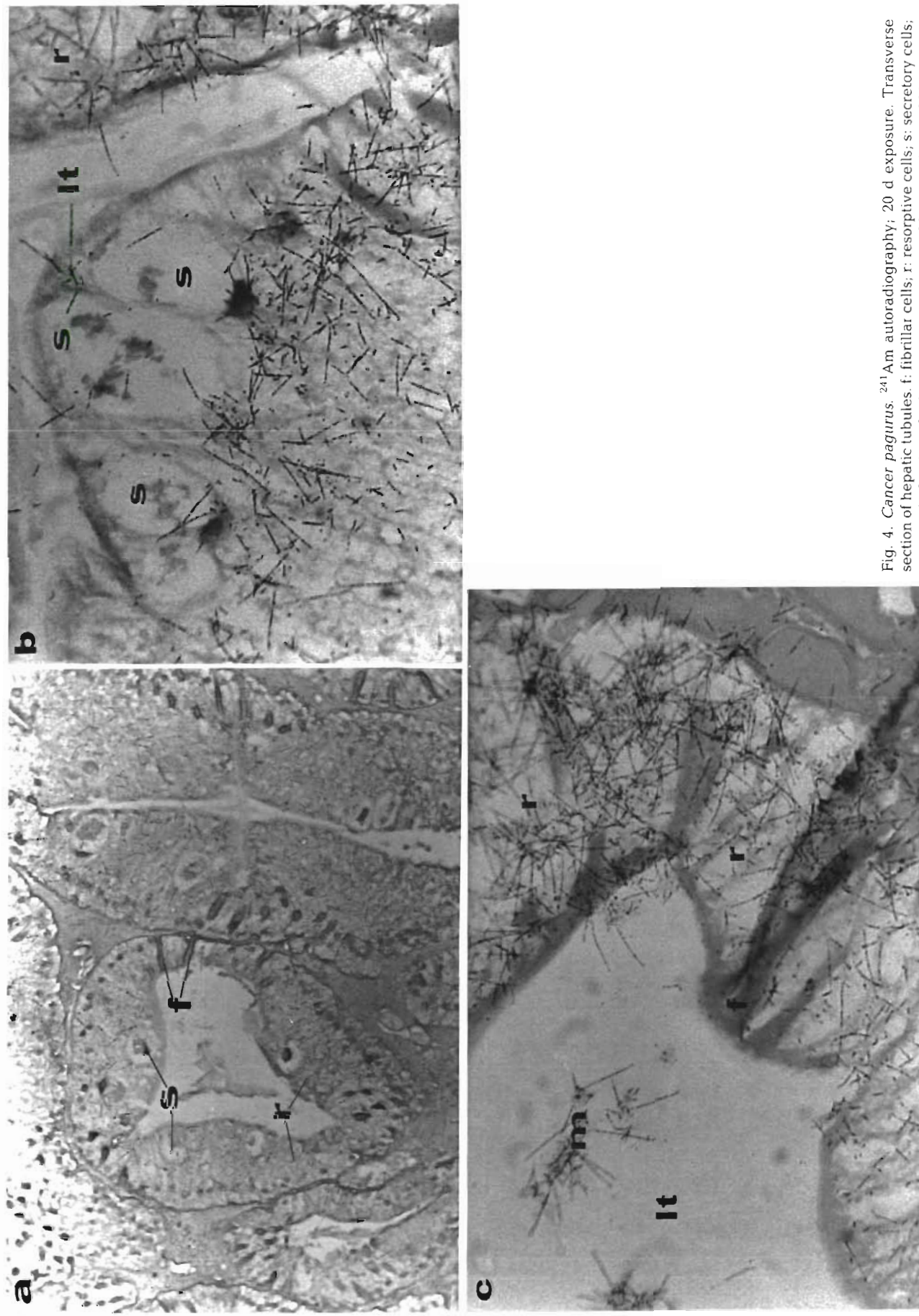

Fig. 4. *Cancer pagurus.* ^{241}Am autoradiography; 20 d exposure. Transverse section of hepatic tubules. f: fibrillar cells; r: resorptive cells; s: secretory cells; lt: lumina of tubules; m: mucus. (a) × 96; (b) and (c) × 380

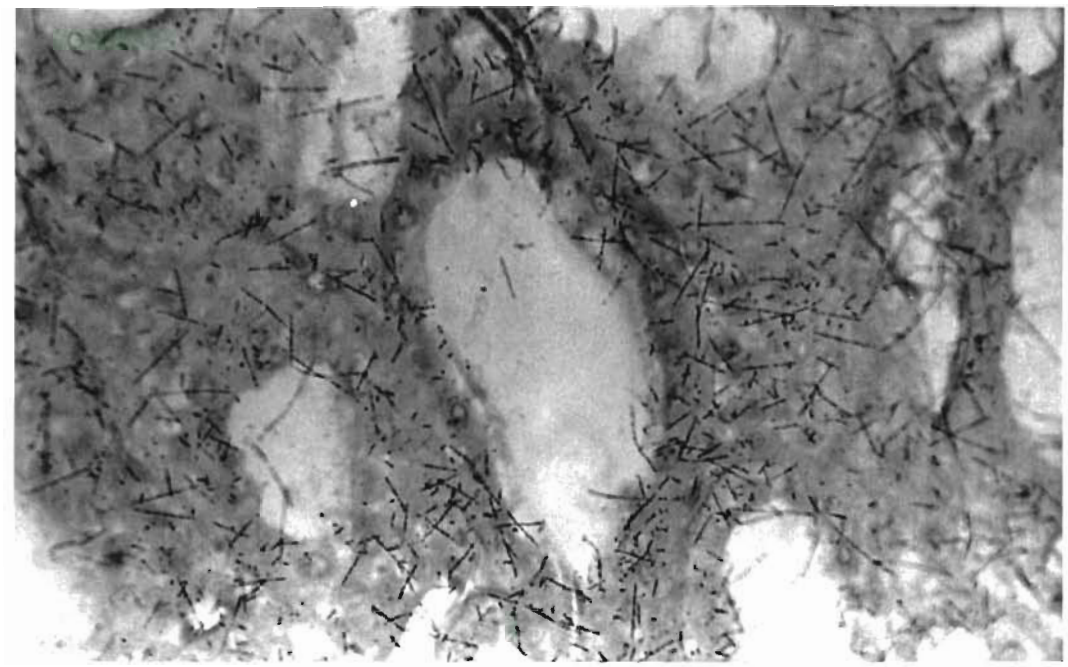

Fig. 5. *Homarus gammarus.* ^{241}Am autoradiography; 21 d exposure. Transverse section of labyrinth of green gland. × 400

those noted for plutonium (Ward 1966, Guary et al. 1976). The marked binding of these 2 elements in the gills of decapod crustaceans is particularly interesting. The covering of these organs is non-calcified and chitinous, and is discarded like the exoskeleton upon moulting. The gills have a predominantly respiratory role, but also have a semi-permeable wall in direct contact with sea water which is involved in ion exchange and osmotic balance. The marked binding of americium in the gills of lobsters and crabs could indicate that they represent an essential site for absorption of transuranic elements present in the surrounding sea water, as for many other elements, notably heavy metals (Bryan 1984). But histo-autoradiography indicated that in lobster, americium was localised in the mucus between the gill lamellae, and in crab, on the chitinous external surface of the gills. The mucus and chitin contain polysaccharides which are known to bind transuranic elements, as has been shown in polychaetes, bivalve molluscs and other crustaceans (Hamilton & Clifton 1980, Grillo et al. 1981, Carvalho & Fowler 1984, Miramand et al. 1987). This localisation indicates that the marked binding of americium in the gills of decapod crustaceans is essentially due to simple adsorption on the large surface area of these tissues, as in the exoskeleton. The localisation of americium is identical to that seen with iron, which is adsorbed by the exoskeleton in various decapod crustaceans and forms a coating around the gill lamellae (Martin 1975). Martin (1975) has also shown in the lobster *Homarus americanus* that the gills, the structure of which differs from those of the brachyuran decapods *Carcinus maenas* and *Cancer irroratus,* do not play a filtering role, and hence bind much less iron from sea water than the gills of crabs. In our study, we also note that americium adsorption is greater in the gills of brachyuran decapods than in those of macruran decapods: CF ≈ 700 for the crab, CF ≈ 130 for the lobster.

Besides this extensive physicochemical adsorption of americium on the gills and exoskeleton, non-negligible penetration into the internal tissues was also noted. The highest concentration factor in the soft part of the lobster was noted for the epidermis (CF ≈ 79), and was 6- to 7-fold higher than that seen in the hepatopancreas (CF ≈ 12). The same ratio was noted by Guary (1980) in the crab *Carcinus maenas* after 8 d uptake of americium from sea water (CF ≈ 40 for the epidermis, CF ≈ 5 for the hepatopancreas). This fact is important since it should be compared with exoskeletal permeability and possible transcuticular transfer of americium from the sea water to the epidermis. It is true that the possibility of simultaneous internal transfer from the haemolymph to the epidermis cannot be totally excluded, but it is certainly less marked.

The hepatopancreas in decapod crustaceans is the organ of digestion, absorption and storage of nutrients, notably carbohydrates and lipids (Gibson & Barker 1979) and numerous metal ions (Bryan 1984). After a relatively short labelling period, the concentration factors for americium in the hepatopancreas of lobster and crab were 12 and 7 respectively. After a longer period of uptake (220 d), Ward (1966) noted a concentration

factor of about 100 for plutonium in the hepatopancreas of the European lobster, a value close to those noted for the exoskeleton and gills at the end of the experiment. Ward's data, together with the results presented here, confirm the important role of the hepatopancreas in the transfer of transuranic elements in decapod crustaceans when the contaminating vector is sea water.

The localisation of americium in the hepatopancreas is essentially intracellular in the lobster and crab. Guary & Negrel (1981) studied the hepatopancreas of the crab using nutrients labelled with americium, and after subcellular fractionation of this organ they observed americium in the centrifugation pellet containing microgranules of calcium phosphate. We have also detected americium in various cells of the hepatopancreas of the crab, notably in the resorptive cells which contain these microgranules. We have also noted americium in the same cell types in the lobster, in which these microgranules are absent (Gibson & Barker 1979). This gives reason to believe that retention of americium involves other processes in the digestive gland of decapod crustaceans, and further studies are required to clarify this point. In any case, americium, which is an artificial element without any known metabolic role, penetrates the cells of the digestive gland.

Several transfer pathways are possible. Transfer from sea water to the canaliculi of the digestive gland is highly likely. It can be imagined that the americium absorbed with sea water is metabolised by the digestive system in the crab and lobster. Thus in crabs, Fowler & Guary (1977) have demonstrated marked assimilation of plutonium contained in food. The preponderant role of the digestive tract in uptake of americium and plutonium in sea water has also been noted in a bivalve, the cockle *Cerastoderma edule* (Miramand & Germain 1985). However, it remains possible that americium is transferred to the hepatopancreas, with transport by the haemolymph from the gills or from the epidermis. This point requires further clarification.

The presence of americium in the cells of the green gland indicates obligatory transport of this element by the haemolymph. Our study has shown that the green gland, the excretory gland of decapod crustaceans, is involved in elimination of americium. A previous experiment performed in our laboratory (unpubl.) confirms this. The green gland of lobsters following accumulation of ^{241}Am by the whole animal from sea water showed greater activity per unit mass 56 d after the end of the labelling than the green gland of lobsters dissected at the end of the uptake period. This implies that the presence of americium in the green gland is due to transfer from the hepatopancreas. In vitro experiments in the crab have demonstrated that plutonium is associated with a haemolymph metalloprotein, which could be haemocyanin or one of its subunits (Guary & Negrel 1980). A vector for transport of transuranic elements in the haemolymph may be involved. These transfers must, however, be very rapid since its has never been possible to demonstrate very high concentrations of transuranic elements in the haemolymph of decapod crustaceans (Guary 1980).

Complementary studies are under way in our laboratory to specify the mechanisms of transfer of transuranic elements in crabs and lobsters, and to evaluate the relative importance of each mechanism.

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This article was presented by Dr J. Mauchline, Oban, Scotland

Manuscript received: September 18, 1988
Revised version accepted: January 18, 1989