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EMISSION AND X RAY SPECTROMETRY**

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► **To cite this version:**

C. Chassard-Bouchaud, F. Escaig. UPTAKE, STORAGE AND EXCRETION OF URANIUM BY MYTILUS EDULIS, A STRUCTURAL, ULTRASTRUCTURAL AND MICROANALYTICAL STUDY BY SECONDARY ION EMISSION AND X RAY SPECTROMETRY. *Journal de Physique Colloques*, 1984, 45 (C2), pp.C2-545-C2-548. 10.1051/jphyscol:19842124 . jpa-00223793

HAL Id: jpa-00223793

<https://hal.science/jpa-00223793>

Submitted on 4 Feb 2008

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UPTAKE, STORAGE AND EXCRETION OF URANIUM BY MYTILUS EDULIS.
A STRUCTURAL, ULTRASTRUCTURAL AND MICROANALYTICAL STUDY BY SECONDARY
ION EMISSION AND X RAY SPECTROMETRY

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RESUME - 1-Deux méthodes de microanalyse ont été utilisées: la spectrographie des rayons X à l'échelon des microscopes optique et électronique et l'émission ionique secondaire à l'échelon de la microscopie optique; elles ont permis de montrer que, des M. edulis récoltées sur les côtes de la Manche, ainsi que des échantillons contaminés expérimentalement, accumulent 238U.

2-Les plus fortes concentrations d'uranium sont détectées dans les animaux prélevés au niveau des zones de rejet des phosphogypses et des résidus de fabrication de TiO₂ qui contiennent de l'uranium.

3-L'uranium est absorbé par les voies branchiale et digestive. Le stockage est réalisé dans le manteau, la glande digestive, l'épithélium intestinal et la gonade où les teneurs les plus élevées sont souvent constatées. L'excrétion se fait par le rein.

4-Les organites cibles sont les lysosomes de la glande digestive et les sphérocristaux du rein; dans ces deux sites électifs d'accumulation, l'uranium est toujours associé à du phosphore. Ainsi, l'uranium absorbé sous forme soluble, est ensuite concentré sous forme de phosphate insoluble dans les organites de stockage.

5-Les hémocytes macrophages jouent un rôle important dans la capture, le transport, le stockage et l'excrétion du radionucléide.

6-La Moule commune apparaît comme un système biologique capable d'accumuler et de concentrer, sous forme insoluble, l'uranium présent dans le milieu marin à l'état de traces. M. edulis est proposé comme organisme indicateur de pollution par l'uranium.

ABSTRACT-1-Two microanalytical methods have been used: X ray emission at the light and electron microscope levels and secondary ion emission at the light microscope level. They allowed to show that M. edulis collected on the Channel coasts or experimentally contaminated samples, bioaccumulate 238U.

2-The highest U levels were detected in the samples collected in the areas where phosphogypsum and TiO₂ industrial wastes, U containing, are released.

3-Uranium uptake happened via gill and digestive tractus. Storage organs were mantle, digestive gland, intestine epithelium and gonad where the highest values often occurred. Excretion happened via kidney.

4-The target organelles were digestive gland lysosomes and kidney spherocrystals: within both of these accumulation sites, uranium was always associated with phosphorus. Thus, soluble uranium which was absorbed, was then concentrated in the form of an insoluble phosphate in the storage organelles.

5-Macrophage haemocytes played an important part in ingestion, transport, storage and excretion of the radionuclide.

6-The common marine Mussel, appears as a biological system accumulating uranium which, present in the marine environment at trace level, is stored and concentrated under an insoluble form by the Mussel. M. edulis is proposed as an uranium pollution indicator organism.

The marine Mussel is of interest, as it is a sedentary filter feeding organism, which is likely to accumulate pollutants such as radionuclides which are released into the marine environment. The increasing use indeed of uranium by chemical and nuclear in-

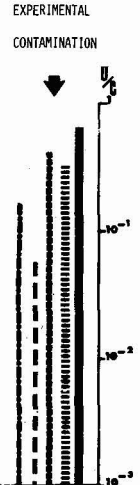
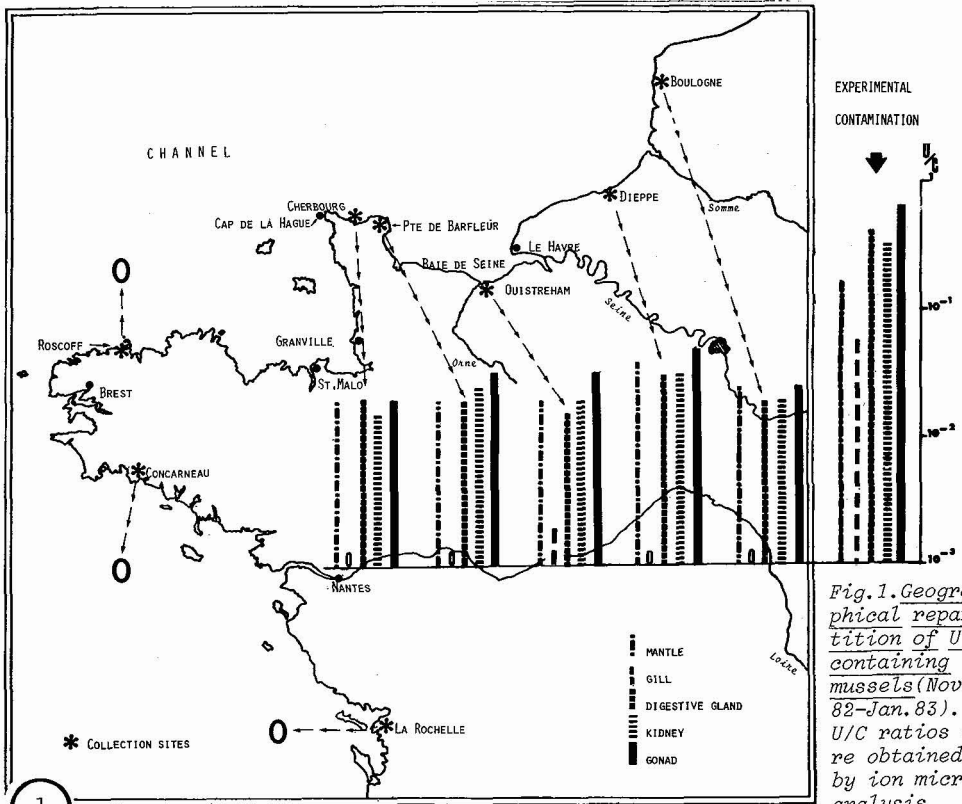
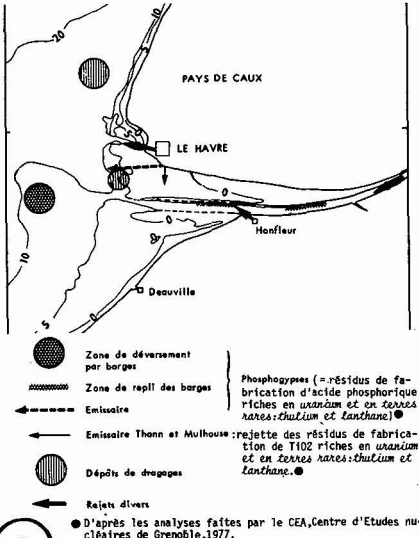
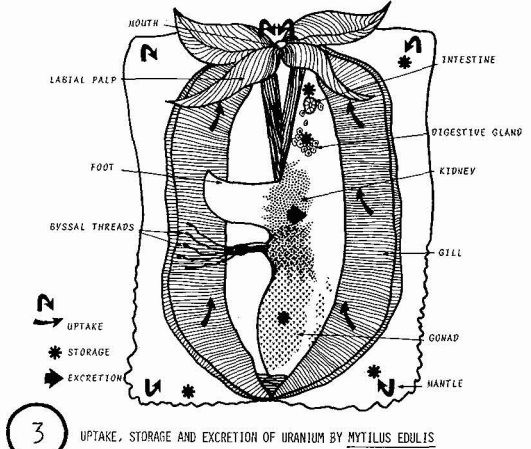


Fig.1. Geographical repartition of U containing mussels (Nov. 82-Jan. 83). U/C ratios were obtained by ion micro analysis.

1 URANIUM BIOACCUMULATION BY THE MUSSEL MYTILUS EDULIS



2 Nature et localisation des rejets affectant l'estuaire de la baie de Seine orientale. (D'après O. BOUST, thèse de 3ème Cycle, 1981: les métaux traces dans l'estuaire de la Seine et ses abords, CEA, Laboratoire de Radioécologie marine de Cherbourg)



3 UPTAKE, STORAGE AND EXCRETION OF URANIUM BY MYTILUS EDULIS

Fig.3. Schematic representation of the part played by the different organs.

dustries and its possible discharge into the aquatic environment give an ecotoxicological interest to the radionuclide. Uranium is a heavy metal which consists of 3 isotopes ^{238}U (99,3 %), ^{235}U (0,7 %) and ^{234}U (0,006 %), all α emitters, chemotoxic and radiotoxic. Recent microanalytical studies, performed on sea and freshwater Crustacea (1)(2), provided the first data on uranium metabolism in aquatic organisms. As nothing was known about the uranium metabolism in Mollusca, our purpose was to investigate about it, on a species of economical interest: *Mytilus edulis*.

1-MATERIALS. Samples of mature *M. edulis* (L) (Mollusca Lamellibranchiata), were collected from November 1982 to January 1983 in 6 stations of the French coasts of the Channel: Boulogne, Dieppe, Ouistreham, Pointe de Barfleur, Cherbourg and Roscoff. The Baie de Seine is an area where phosphogypsum and TiO_2 industrial wastes, U containing, are released. For comparison, other samples were collected from 2 stations of the French coasts of the Atlantic Ocean: Concarneau and La Rochelle.

For experimental contamination, U free mussels were used. They were exposed to an uranium nitrate solution at a concentration of 10 ppm, during a period from 1 to 30 days.

2-SAMPLES PREPARATION AND MICROANALYTICAL METHODS. Cf the other paper of the same author, in the same Congress Proceedings (3).

3-RESULTS.

Mussels collected "in situ" from the Channel and mussels experimentally contaminated were shown to concentrate Uranium.

3-1- Secondary ion emission microanalysis.

$^{238}\text{U}^+$ values normalized to $^{12}\text{C}^+$ are given in fig. 1 for samples of every collection site; they point out the geographical repartition of uranium bioaccumulation in mussels collected from the Channel. This map shows that the highest values were detected in the eastern samples collected from Cherbourg to Boulogne. No uranium was detected neither in Roscoff samples nor in the Atlantic Ocean ones.

The experimental contamination results (fig. 1) show that uranium was detected, in order of decreasing concentration, in gonad, digestive gland, kidney, mantle and gill. These results are in agreement with the "in situ" samples results. Ion images obtained from several organs and tissues show the uranium distribution (fig. 4, 5, 6 and 7).

3-2- X ray emission microanalysis.

3-2-1-Light microscope level: spectra obtained on the MS46 microprobe confirm the secondary ion emission microanalysis data.

3-2-2-Electron microscope level: electron dense microneedles were observed within the digestive gland lysosomes (fig. 8) They were shown to contain high levels of uranium associated with phosphorus (fig. 9). Moreover, intra and extracellular spherocrystals (fig. 11) containing also U and P were observed in the kidney. Macrophage haemocytes, which occur within many organs and tissues of the mussel, exhibited many uranium containing inclusions (fig. 10). They play an important part in ingestion, transport, storage and excretion of the radionuclide.

4-DISCUSSION AND CONCLUSION.

Uranium was detected in the samples collected in the areas where phosphogypsum and TiO_2 industrial wastes, U containing, are released. These results were obtained from mussels collected from November 1982 to January 1983.

Fig. 3 gives a schematic representation of the involved mechanisms by which *M. edulis* is supposed to assume uranium uptake, storage and excretion. Detoxication processes happen via kidney from which the spherocrystals are released into the extracellular medium and via the macrophages. Uranium uptake, concentration and elimination mechanisms above described at the ultrastructural level, for the Mussel, may be compared to those previously described for Mammals (4) and for Crustacea (1)(2).

As *Mytilus edulis* bioaccumulates uranium, present in the marine environment at trace level, it is then proposed to use it as an indicator of uranium pollution.

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(2) CHASSARD-BOUCHAUD C., Mar. Poll. Bull., 14, 4, (1983), 133.

(3) CHASSARD-BOUCHAUD C., HALLEGOT P. and MEIGNAN M.

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Sources of partial support for this work were CNRS (GRECO MANCHE) and INSERM (SC27). The technical assistance was provided by F. Kleinbauer and S. Halpern. Material was, in part, collected by D. Calmet.

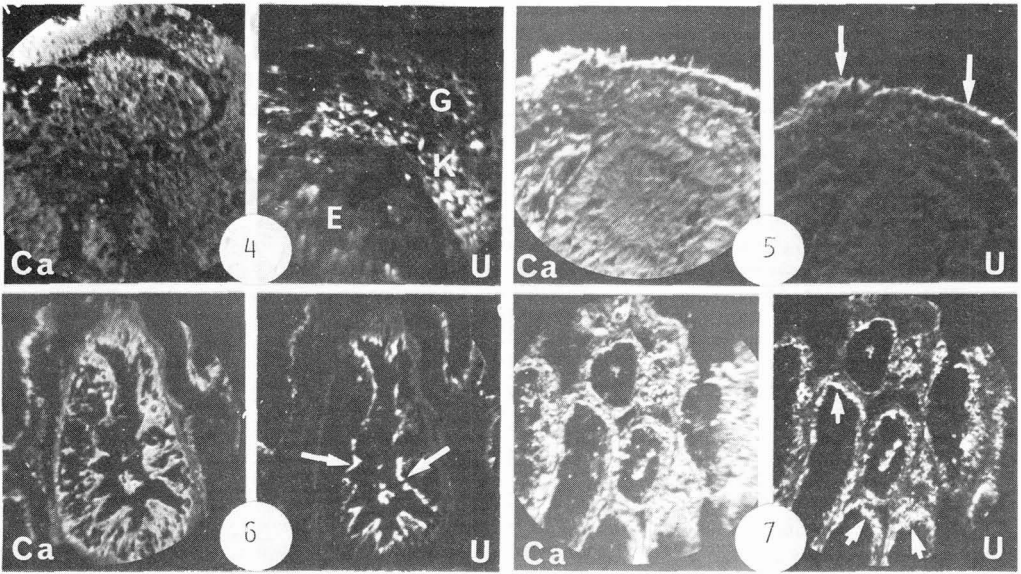


Fig. 4 to 7: calcium and uranium images of every figure were obtained from the same section. (MX 150). Fig. 4: Mantle section. U is present in the gonad(G), the epithelial cells (E) and in the kidney(K). Fig. 5: Mantle section. U is concentrated on the border. Fig. 6: Intestine section: U is concentrated in the absorptive border (arrows). Fig. 7: digestive gland diverticula section: U is accumulated within the cells (arrows).

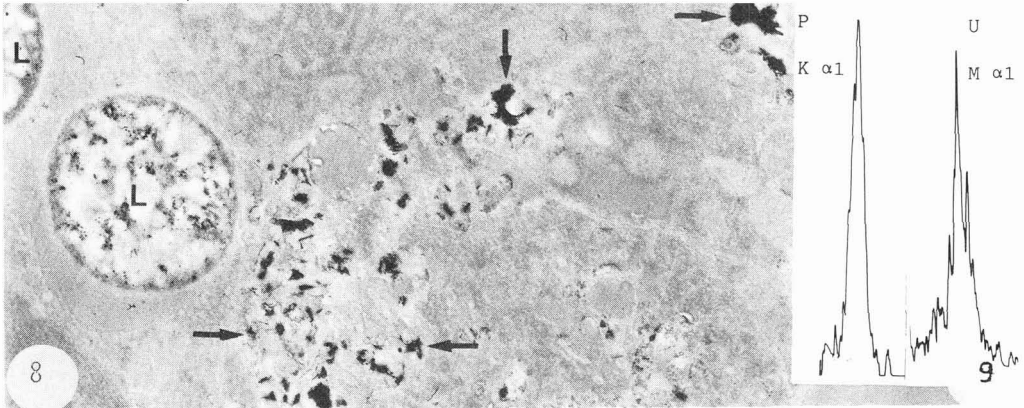


Fig. 8. U concentration in the digestive gland lysosomes (L) which contain microneedles of U associated with P as shown by the spectra obtained (fig. 9). Some lysosome membranes have more or less disappeared (arrows) (M X 15,000). non osmicated and unstained

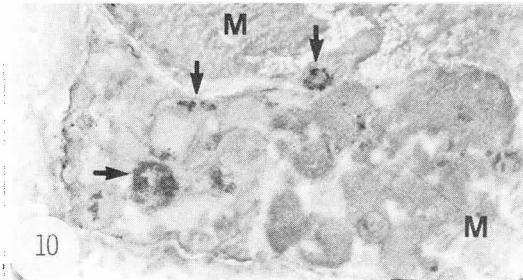


Fig. 10. 2 macrophage haemocytes (M) containing U inclusions (arrows) (M X 5000). Non osmicated and unstained.

Fig. 11. Kidney spherocrystal containing U & P Non osmicated and unstained.

