IDENTIFICATION OF TIEMANNITE AS A PROBABLE PRODUCT OF DEMETHYLATION OF MERCURY BY SELENIUM IN CETACEANS. A COMPLEMENT TO THE SCHEME OF THE BIOLOGICAL CYCLE OF MERCURY

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MERCURE
SELENIUM
TIEMANNITE
DETOXICATION
CÉTACÉS

RÉSUMÉ. – Dans le foie des Cétacés, les concentrations élevées en mercure minéral et en sélénium, ainsi que la corrélation entre les teneurs en ces deux éléments, s'expliquent par la présence d'inclusions de tiemannite (séléniure mercurique cristallisé). La cristallisation de tiemannite pourrait représenter la phase terminale d'un processus de déméthylation du mercure par le sélénium. L'apparition, dans une chaîne trophique, d'un composé non biodégradable du mercure, nous conduit à proposer un complément au schéma du cycle de ce métal dans la biosphère.

MERCURY
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ABSTRACT. – In Mediterranean cetaceans, particles of pure tiemannite are stored in the connective tissue of the liver. The biosynthesis of tiemannite could be the last stage of detoxification process leading to the fossilization of mercury and selenium under the form of a non biodegradable component. A complement to the scheme of the biological cycle of mercury is suggested.

A high proportion of the total concentration of mercury in marine contaminated animals occurs as methylmercury, which is the more toxic form of the metal. Methylmercury is concentrated in the brain, flesh and viscera of fishes and mammals (Koeman *et al.*, 1973; Westöö, 1973), while some organs are characterized by a high proportion of inorganic mercury (Rivers *et al.*, 1972; Freeman and Horne, 1973; Koeman *et al.*, 1973); therefore, a demethylation leading to inorganic mercury is considered (Bryan, 1976). In the liver of seals, porpoises and dolphins, the concentration of mercury is clearly correlated with the concentration of selenium (Koeman *et al.*, 1973); it has been suggested that selenium could have, in marine animals as well as in rats, quails or tissue cultures studied in laboratories, a protective effect against the toxic action of mercuric compounds (Parizek and Ostadalva, 1967; Ganther *et al.*, 1972; Iwata *et al.*, 1973; Moffitt and Clary, 1974; Stoewsand *et al.*, 1974; Ohi *et al.*, 1975; Potter and Matrone, 1977); selenium and mercury could be associated together with proteins by means of sulphur (Parizek *et al.*, 1969; Ganther *et al.*, 1972; Koeman *et al.*, 1973).

We have studied cetaceans captured in a very polluted area, the Mediterranean sea. In these animals, analyses of numerous organs reported by Viale (1976, 1977) show that most of the mercury is stored in the flesh and the liver; the fraction of methylated mercury is important in all organs (25 to 70 %) except in the liver (3 to 7 %). Histological survey of organs of *Ziphius* and *Tursiops* shows in the liver alone black particles (1 to 5 μm), irregular in shape, located in the connective tissue of the portal vessels (Martoja and Viale, 1977). In ultrathin sections, each particle appears to be composed of numerous electron-dense granules measuring about 15 nm (Plate I, A, C). They are not digested by proteolytic enzymes (pronase, pepsine, trypsine). Only two elements, Se and Hg, are detected in them with the electron microprobe (Fig. 1). Quantitative X-ray microanalysis of the particles was made with crystal spectrometers and by comparing the ratio $(P\text{-}b)_{Hg}/(P\text{-}b)_{Se}$ obtained for particles with those given by a commercial standard of pure mercuric selenide HgSe (P = peak, b = back-ground). The ratios ($2{,}66 \pm 0{,}44$ for particles and $2{,}57 \pm 0{,}34$ for standard) do not differ significantly and hence, the ratio Hg/Se is identical in particles and mercuric selenide. Dried samples of liver of *Ziphius* were analysed for total mercury by flameless atomic absorption spectrophotometry, for methylmercury by gas chromatography and for selenium by colorimetry of the selenium – 3,3 diaminobenzidine hydrochloride complex. The concentration of total mercury was 1 343 mg/kg, while the concentration of selenium was 477 mg/kg. The weight ratio "total Hg/Se" is 2,8, the weight ratio "inorganic Hg/Se" is 2,7, this last value corresponding to a 1/1 molar ratio.

Thus data obtained by histological survey, quantitative X-ray spectrography and chemical analysis are in agreement : selenium and mercury are stored together in characteristic granules, as a product whose molar ratio Hg/Se is 1/1.

Diffraction patterns of particles were obtained by using ultrathin sections of *Ziphius* liver (Plate I, B). Parameters of these patterns are similar to those of a standard crystal of mercuric selenide HgSe (tiamannite). Therefore, particles stored in the liver of Cetaceans are constituted of pure tiemannite.

The storage of mercury and selenium as mercuric selenide explains the correlation, discovered in several marine animals (Koeman *et al.*, 1973), between these two elements. We think that cristallization of tiemannite could be the final stage of a detoxification process which consists in a methyl-transfer from mercury to selenium. Indeed, the food of Cetaceans (fish, sleevefish) is very rich in methylmercury (Viale, 1977), and so inorganic mercury stored in the liver must originate from the organic form of the metal. According to the opinion of

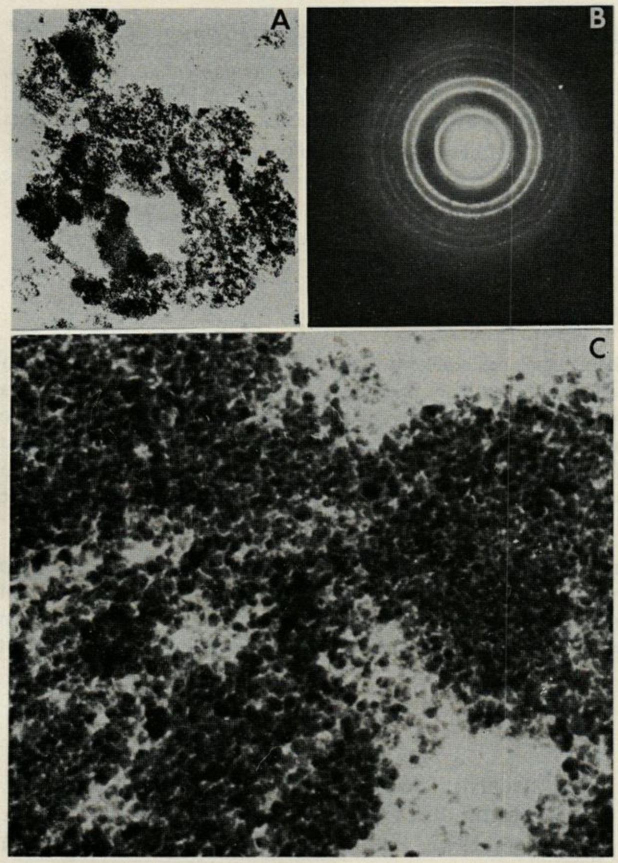

PLANCHE I. – *Ziphius cavirostris* femelle, âgée de plus de 12 ans, capturée en 1974. A : section ultrafine d'un amas granulaire (fixation : formol, sans coloration). Notez les nombreux granules denses aux électrons; B : image de diffraction d'une de ces particules; C : détail au niveau d'une particule montrant la juxtaposition des granules de tiemannite (× 180 000).
Ziphius cavirostris *female, over 12 years old, captured in 1974. A : ultrathin section of a particle (fixation : formaldehyde, no staining). Note the numerous electron-dense granules (× 40,000); B : diffraction pattern of such a particle; C : enlarged view of a portion of a particle showing the juxtaposition of the tiemannite granules (× 180,000).*

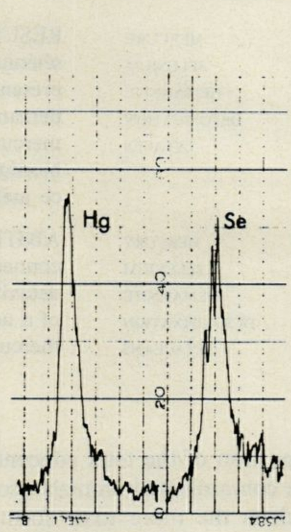

Fig. 1. – *Ziphius cavirostris* âgée de plus de 12 ans. Mise en évidence de Mercure et de Sélénium au sein d'une particule, dans une coupe à la paraffine (7 μm) collée sur une lame de « mylar » carbonée, éclaircie au toluène. Microsonde Cameca MS 46, avec un faisceau (15 kV à 40 nA) dirigée sur 1 point d'environ 1 μm; spectromètre à cristal K.A.P.
Ziphius cavirostris *female, more than 12 years old. Demonstration of mercury and selenium on a particle in a paraffin section (7 μm) glued on a mylar carbon-coated slide and cleared with toluene. Microprobe Cameca MS 46, with a beam (15 kV at 40 nA) focussed into a spot of about 1 μm; spectrometer crystal K.A.P.*

Wood *et al.* (1975), the detoxification of methylmercury poisoned animals by selenium can be explained by a methyl group transfer out of the mercury cycle into the selenium cycle; this transfer requires vitamin B12, and produces dimethylselenide which is ventilated from the lungs, but the fate of demethylated mercury is not considered. Our observations show that cristallization of tiemannite occurs in the liver, in which the level of vitamin B12 is high enough to allow the methyltransfer; dimethylselenide can be carried to the lungs by the vessels, while demethylated mercury reacts with part of the selenium which has been reduced to the lower oxidation stage (2^-).

The detoxification pathway evidently involves several reactions which are still unknown. Our results do not disagree with experimental data that show a liberation of methylmercury from sulfhydril bonds, produced by selenium salts (Sumino *et al.*, 1977): the release of organic mercury from protein linkages could be the first effect of the metalloid. Under natural conditions, the protective effect of selenium could be due to selenious acid present in sea water (Riley, 1975) or to an organic compound supplied by the food (Lunde, 1970). The last stage of the detoxification pathway is not a linkage of mercury and selenium to proteins by means of sulphur; it is also well known that selenium does not complex SH groups but oxidizes them (Jernelov and Martin, 1975). Likewise, the ocurrence of selenoproteins, which have a high affinity for mercury (Fang, 1977) is not evident in the liver of Cetaceans.

An important fact is the impossibility for cetaceans to excrete the tiamannite particles; because these animals live a long time (more than 25 years), the liver of old individuals shows a great number of particles; in a *Ziphius* twelve years old the liver contained about 13 g of inorganic mercury and 5 g of selenium. The biosynthesis of tiemannite is a process leading to the fossilization of mercury and partially of selenium under the form of a non biodegradable component; neither the cetaceans nor any organism of a food chain are able to metabolize again the mercury bound to selenium. It will be necessary to determine whether this peculiar mechanism of detoxification does exist in other zoological groups; it is probably the case at least for species where a 1/1 molar ratio fo Hg and Se suggests a direct Hg-Se linkage (marine mammals, Koeman *et al.*, 1973; cat, Uzioka, 1960; man, Kosta *et al.*, 1975, Kennedy *et al.*, 1977), and for the rat whose phagocytic cells concentrate Se and Hg in aggregates (Groth *et al.*, 1973).

Without claiming the occurrence of such a detoxification process in a wide range of animals, these results indicate that some elements are to be added to the scheme of the mercury cycle in the biosphere, as proposed previously by Wood *et al.*, 1975 (Fig. 2).

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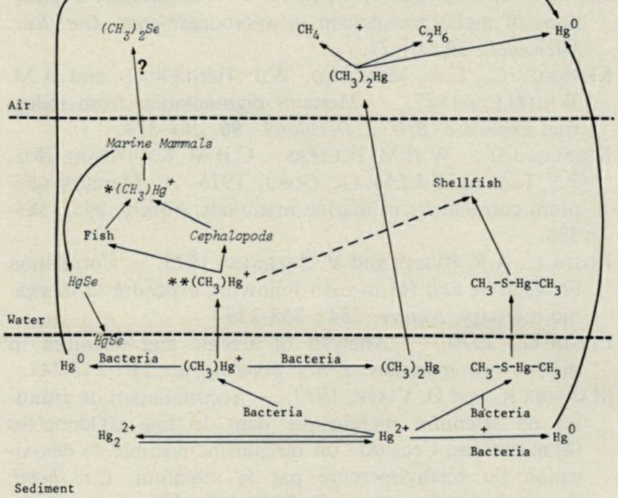

Fig. 2. – Cycle biologique du Mercure, modifié d'après Wood *et al.* (1975). Les lettres en italique sont employées pour de nouvelles données concernant les Céphalopodes et les Mammifères marins (Viale, 1977; Martoja and Berry, cet article), la flèche en pointillés pour les données de Huckabee *et al.* (1975), concernant les Crustacés. ** $(CH_3)Hg^+$: méthyl-mercure de l'eau et de la nourriture (plancton); * $(CH_3)Hg^+$: méthyl-mercure de la nourriture.

*The biological cycle for mercury, modified from Wood et al. (1975). Italic letters are used for new data concerning Cephalopods and marine Mammals (Viale, 1977; Martoja and Berry, present paper), dotted arrow for data by Huckabee et al. (1975) concerning shellfish. ** $(CH_3)Hg^+$: methylmercury in water and food (plankton); * $(CH_3)Hg^+$: methylmercury in food.*

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