

## BIOGEOCHEMISTRY RESEARCH OF FLUORIDE IN ANTARCTIC OCEAN II. THE VARIATION CHARACTERISTICS AND CONCENTRATION CAUSE OF FLUORIDE IN THE CUTICLE OF ANTARCTIC KRILL

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**Abstract** The cause of the concentration of fluoride in Antarctic krill is studied by the analysis of the characteristics of fluoride change in the cuticle of the krill before and after moulting. Associated with other related information, the source and accumulating mechanism of fluoride in krill are also discussed. Results show that as an inorganic medium the cuticle of krill has the second concentrating function and action to fluoride after moulting, which has nothing to do with the biological action of the krill. The fluoride is concentrated from seawater, which is prosecuted mainly in the form of ion exchange.

**Key words** Antarctic krill, concentration cause, fluoride

### Introduction

Since Soevik and Braekkan (1979) found high concentration of fluoride in Antarctic krill, it has become an important problem to explain the cause of fluoride concentration. Although a few studies have been carried out, the conclusion is still not clear. In recent years, some scientists reported their researchs around the high fluoride anomaly of krill. Most of these works are in respect to the homeostasis of fluoride in krill. For instance, Buchhloz (1983) and Adelung (1987) studied the changes in fluoride content during the moulting cycle of krill and suggested that such changes in the cuticle is related to its physiological action. However, does this imply the cause of the fluoride anomaly in the krill?

The authors had preliminarily probed into the fluoride anomaly and its relation with environment (Zhang, 1991). On the bases of this work, the origin, causes and mechanisms of fluoride in krill are studied by analysing the change characteristics of fluoride content in the cuticle before and after moulting, and some new points of view are suggested in this

paper.

### Materials and Methods

Samples are collected from  $60\sim 66^{\circ}\text{S}$ ,  $52\sim 68^{\circ}\text{W}$  and  $60\sim 62^{\circ}\text{S}$ ,  $105\sim 110^{\circ}\text{W}$ , during the 7th Chinese Antarctic Research Expedition.

The living specimen were immediately sectioned into head, chest and cuticle after caught, and quickly dried to preserve. Some other living specimen were kept in seawater by imitating natural condition. Then the cuticle samples of different time order were collected. All samples were brought to laboratory for analysis.

The fluoride in cuticle was all determined by IMS-3F, SIMS(Cameca, Co., France).

### Results and Discussion

#### 1. Dynamical Variation of Fluoride in Cuticle

Fig. 1 expresses the fluorine contents in cuticle of krill before and after moulted.

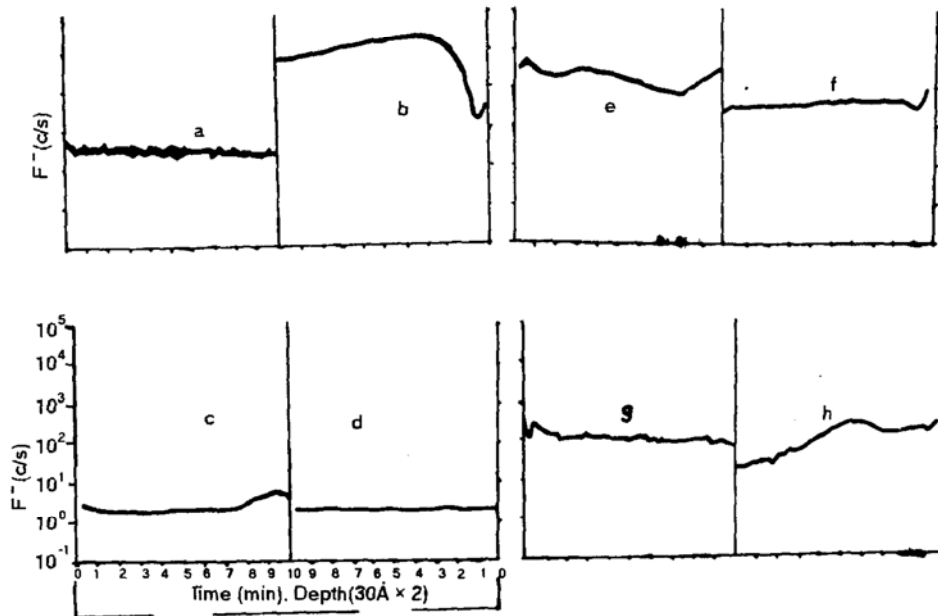


Fig. 1. Comparison of fluoride content between the cuticle and remnant cuticle in krill.

a, b—before the moulting; c, d—after the moulting; e, f—12 hours after the moulting.

The analysing results showed the variation characteristics for fluoride in cuticles and shed moults of krill;

A. Before moulting, the cuticle has a high fluoride content level, but the content distribution is not even. (Fig. 1a, b).

B. While moulting, fluoride in cuticle is expelled and the fluoride content approaches zero in the new cuticle(Fig. 1c,d).

C. After moulting, the fluoride contents in the shed moults get obviously high and it can be as much as twice of the contents before moulting, which shows that the shed moults can still absorb fluoride(Fig. 1e,f).

D. 24 hours after moulting, fluoride content in shed moults is decreased, yet the content is still higher than before moulting(Fig. 1g,h).

E. Before moulting, there is obvious variation in the distribution of fluoride contents through the cuticle depth. The fluoride contents in the interior surface is higher than that in the outer surface. But after moulting, there is no obvious difference.

## 2. The Cause of High Fluoride Anomaly of Krill

Buchholz(1982) and Adelung *et al.* (1987) studied the variation of fluoride content in the cuticle during moulting. Results showed that there was obvious relation between the fluoride content and the physiological change in the moulting—cycle process of krill(Fig. 2).

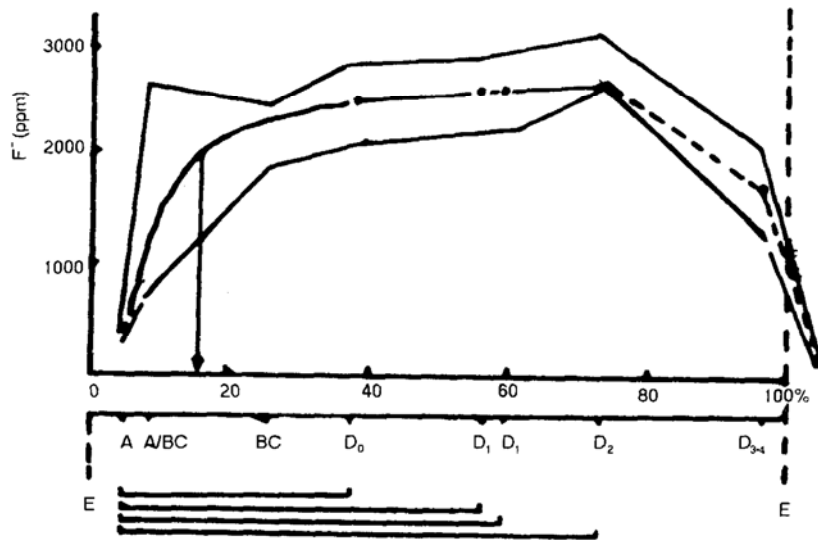


Fig. 2. Fluoride contents of cuticle in moulting stage.

Fluoride content appears lower in the first stage after moulting (Fig. 2, A — A/BC stage) and increase linearly. In the stage of BC—D<sub>2</sub>, fluoride content is much higher and stable. In this stage, the main characteristics of the physiological change is that the cuticle has been harden. However, in D<sub>2</sub>—D<sub>3-4</sub> stage, the content of fluoride decreases obviously. Microanalysis results show that the physiological characteristics of the cuticle in this stage is similar to that in A—A/BC, and all of they are in softing stages. The difference is that the former is from hard to soft while the latter is sharply opposite. From Fig. 2 fluoride variation characteristic in krill's moulting — cycle process, some researchers think that fluoride

concentration in krill is an active absorbing process, that is, fluoride takes part in the biological process of krill, and to be used mainly to hardening cuticle (Buchholz, 1983).

Element concentration in organisms can be divided into active and passive ways. The former is necessary for their life and latter is resulted from the environmental change or other unusual chemical and physical factors, such as the concentration of pollution. According to the results from Adelung *et al.* (1987), the absorption of krill to fluoride should belong to the former. However, the research results related to the variation characteristics of fluoride content in cuticle and shed cuticle before and after moulting demonstrate that the absorption to fluoride in krill is not simply so.

The results in Fig. 1 shows that the fluoride content in cuticle of krill has a obvious discrepancy in short time before and after moulting. Fluoride in cuticle is strongly expelled to go back into environment (seawater), resulting in a fluoride content approaches zero. It seems that the fluoride content is somewhat related to the biological process of krill. however, it deserved attention that the fluoride content in shed cuticle is still not low, the concentration coming up to as high as two times before moulting shed moults (Fig. 1e, f). Though the content of fluoride is decreased after 24 hours, the content is still not lower than that before moulting. This phenomenon fully show that the cuticle can still absorb fluoride strongly after moulting, namely, it has the second absorption function. These evidences show that the concentration of fluoride in krill does not practice only by its biological process.

The cuticle as a special material absorbing fluoride may be one of the causes of concentrating fluoride.

### 3. Origin and Mechanism of Fluoride Concentration in Krill

Fig. 1a, b show that fluoride distribution within cuticle displays a characteristics which fluoride mobilized from the outer surface to interior surface (Fig. 1a), but further observation indicates that just near the interior surface, fluoride content gets rapidly decreased, resulting in the same level of fluoride content in both sides of the cuticle. This shows that, although the cuticle plays a mainly part on the concentration of fluoride, fluoride does not get into soft tissues from carapaces. This conclusion can also be given from Table. 1 which shows that the fluoride content keeps constant invarious soft tissues of krill in moulting—cycle stage. Especially, in the alternate stage of moulting—cycle there no fluoride imported from cuticle to soft tissues.

The concentration of fluoride in krill has not only a high ratio but also a rapid rate. It generally reaches the equilibrium within 36 hours after moulting (Schenppengeim, 1980). Physiological observation indicated that the mouth of krill has not been hard enough for eating in this stage. Supposing the eating can still be carried out, the fluoride content in phytoplantons as the main foods of krill is also not high (Zhang, 1991). Therefore, it can be recognized that the concentration of fluoride in krill should be from seawater and the food

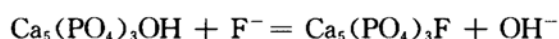
sources be excluded. The high content of fluoride in cuticle seems to indicate that chitin constitute might play an important role in the concentration of fluoride. However, the analysis results (Zhang, 1991) indicated that the fluoride content is not high in chitin ( $200\mu\text{g/g, d. w}$ ), which only accounts for 4.9% of the total amount. This shows that it is impossible for chitin to concentrate fluoride. There must exist other combination forms in cuticle.

Table. 1 Fluoride contents( $\mu\text{g/g, d. w}$ ) in tissue of krill in moulting stage\*.

Samples	Moulting Stages					
	A	BC	D <sub>0</sub>	D <sub>1</sub>	D <sub>2</sub>	D <sub>3-4</sub>
Muscle	5.0	3.7	—	4.3	4.4	4.8
Hemolymph	3.1	4.3	3.9	3.8	5.7	7.9

\* Adelung *et al.*, 1987.

The immigration of fluoride is affected by many factors in environment. In the exogenic geochemical process, absorption is an essential and effective way for fluoride in environment. Generally absorption to fluoride in air is mainly in the way of molecule absorption. However, ion exchange, especially that between  $\text{F}^-$  and  $\text{OH}^-$ , is the major form of absorbing fluoride in solution system. In this process, as a good absorptive agent, the minerals (compounds) composed of calcium and phosphorous play an important part for the concentration of fluoride. Many results have demonstrated that fluoride has closely relation to minerals (compounds) with  $\text{OH}^-$ . Fluoride can replace the ion such as  $\text{OH}^-$  in these minerals and produce other minerals forms. The process could be described by following:



where  $[\text{Ca}_5(\text{PO}_4)_3\text{F}]$  produced by the reaction is the result of replacing  $\text{OH}^-$  in  $\text{Ca}_5(\text{PO}_4)_3\text{OH}$  by  $\text{F}^-$  in the basic medium (seawater). The solubility product of  $[\text{Ca}_5(\text{PO}_4)_3\text{F}]$  is lower than  $2 \times 10^{-51}$ . That is the most stable and major mineral of fluoride in environment. according to the relation fluoride to calcium and phosphorous in the cuticle of krill (Zhang, 1991), it is possible that the combination fluoride with calcium and phosphorous is the main way of concentration in cuticle before and after moulting. As for the rapid lose of fluoride in cuticle during moulting. One possible explanation is the enzymatic action, which results in the destory of cuticle struction as well as the losing fluoride, so as to soft cuticles and carry out the moulting (Adelung *et al.*, 1987). But our observation shows that the hardness of the shed moults will be higher than that before moulting at least within 24 hours. This evidence also proved the possibility of Equ. 1 as the way of the cuticle to concentrating fluoride. Since it has been well known fact that the combination of fluoride with calcium and phosphorous to be a hardener.

### Conclusions

A. The cuticle has a second concentrating function to fluoride. The cuticle of krill as an inorganic medium strongly absorbing fluoride is one of the causes of fluoride concentration in krill.

B. The ion exchange and the association of fluoride with calcium and phosphorous are the most possible ways of the fluoride concentration in cuticle.

C. Fluoride in cuticle indicates the variation characteristics of immigrating from the outer to the interior.

D. The concentration of fluoride in cuticle comes mainly from seawater.

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