Effects of Light, Magnesium, and Cyanide on Accumulation of Mercury by a Fresh Water Diatom, *Synedra*

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It has been reported that mercury was apparently accumulated in fishes and aquatic animals through food chains and/or by direct adsorption from surrounding water through the gill and epitherium, and that its concentration in the flesh reached to levels several thousand times as high as those in the surrounding water. However, little has been known about which route may play a major role in its accumulation. In the previous report, we (FUJITA and HASHIZUME, 1975a) studied the accumulation of mercury into phytoplankton which was known to form a basis of the food chain, intending to determine the extent of contribution of food chain in the accumulation of mercury. The results we obtained have indicated that planktons took up mercury compounds from surrounding water efficiently and rapidly, and that the food chain plays an important role in transferring mercury to organisms at upper trophic levels. Thus, it seems to be very important to elucidate the mechanism of the accumulation of mercuric compounds by planktons.

GLOOSCHENKO (1971), FILLIP and LYNN (1972), and MATSUI and GLOYNA (1972) studied on the mechanism of the uptake of mercury into planktons by the use of laboratory cultures of *Chaetoceros costatum*, *Selenastum capricornutum* and *Chlorella pyrenoidosa*, respectively, and showed that mercury was accumulated in these planktons mainly by passive adsorption. WINDOM et al (1973) have also suggested by determining the level of mercury in North Atlantic planktons that mercury was accumulated in planktons by passive adsorption rather than active uptake. Among these authors, however, GLOOSCHENKO has noticed the presence of the active uptake in *Ch. costatum*, in addition to surface adsorption which appeared to be the most important process in accumulation of mercury in the palnktons. On the contrary, as reported in the previous paper (FUJITA and HASHIZUME, 1975a), we have observed that about 50 percent of the mercury taken up by planktons was found in the inner part of cells. This result strongly suggested that mechanisms other than the passive adsorption were involved in the process of the uptake of mercury by planktons. The purpose of this investigation was to clarify whether or not the biological process participated in the uptake of mercury by planktons.

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Materials and Methods

Synedra ulna var.*danica* was isolated and cultured in the same manner as previously described (FUJITA and HASHIZUME, 1975b) using CHU (1942) No. 16 medium which contains 0.3mM $Ca(NO_3)_2$, 0.075mM K_2HPO_4, 0.16mM $MgSO_4$, 0.16mM K_2SiO_3, 0.1mM $CaCl_2$, 0.19mM Na_2CO_3 and 0.006mM $FeCl_3$. In some experiments, a buffer solution containing 10mM HEPES-HCl* pH 7.0, 0.19mM CO_3^{--} and 0.3mM NO_3^- (standard buffer) was also used as a culture medium.

The uptake of mercury was determined as follows: the cell suspension was incubated with [^{203}Hg]mercuric chloride (1.5mCi/mg ; Radiochemical Centre, England) for the time indicated in appropriate legends. The final concentration of mercury was chosen so that the growth of *Synedra* was not inhibited. After incubation, aliquots of the culture were removed and filtered through a glass fibre filter (Whatmann GF-83, pore size 1 μ) to collect the cells. The filter was washed 4 times either with 5 ml of 0.1mM cysteine solution (pH 6.5) in the case of measuring the mercury uptake into the cells or with 5 ml of culture medium in the case of determining the total uptake including the surface adsorption. The radioactivity retained on the filters was determined as described previously (FUJITA and HASHIZUME, 1975b).

In experiments to study the requirements for the Hg uptake, the cells were harvested at log-phase, throughly washed with distilled water, and resuspended in a standard buffer with or without various ions to be tested at a population size of 1,000-5,000 cells per ml.

Cells which were multiplying under illumination, those which were kept in the dark for 24 hours prior to each experiment to halt the cell division, and those which had been killed by heating at 60°C for 3 minutes will be referred to as dividing, non-dividing and heat-treated cells, respectively.

Results and Discussion

Uptake of mercury by dividing, non-dividing or heat-treated cells

After incubating dividing cells as well as heat-treated cells under illumination and non-dividing cells in the dark in the presence of radioactive mercury, the amount of mercury taken up into these cells was determined as described in Materials and Methods. The results obtained are shown in Figure 1, in which the total uptake, including surface adsorption, is illustrated in solid lines and the uptake into the cells is shown in broken lines.

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*Abbreviation ; HEPES, N-2-hydroxyethyl biperazine-N-2-ethane-sulfonic acid.

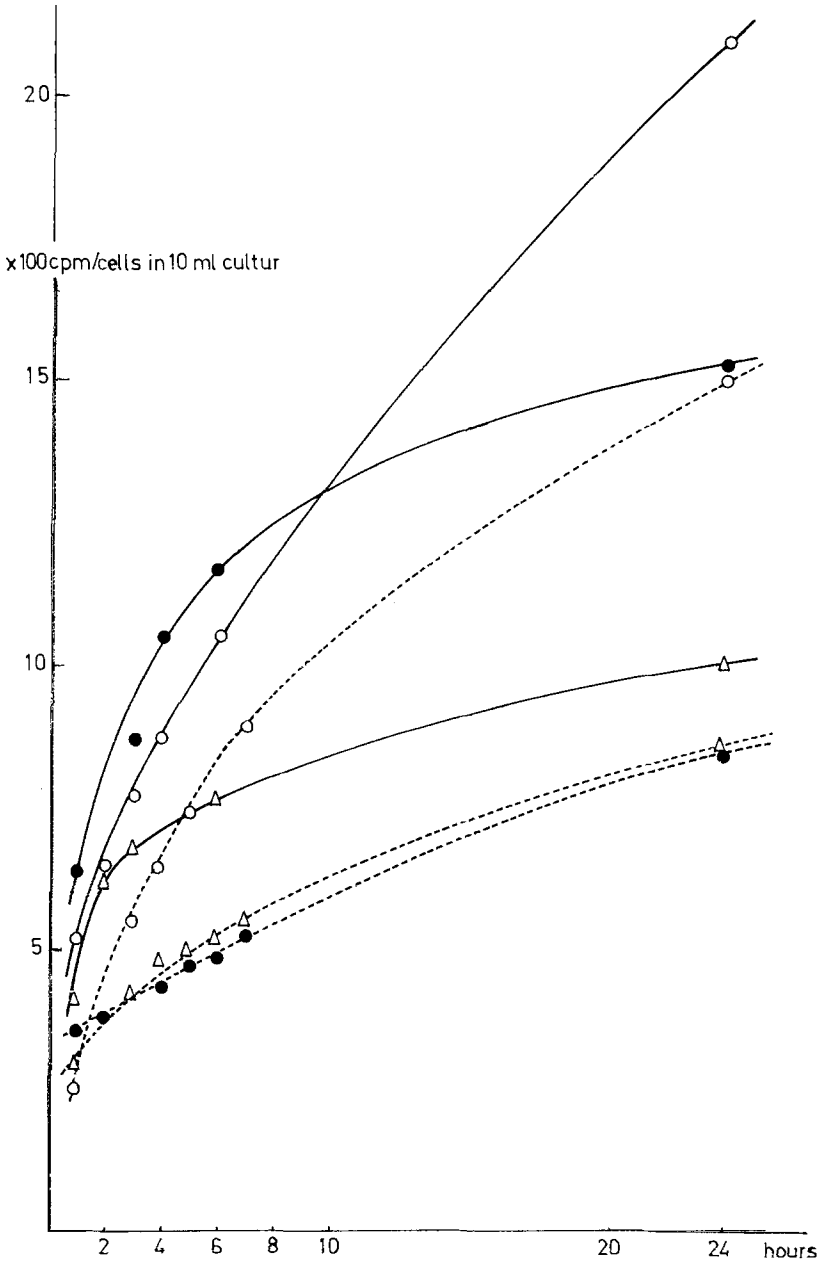

Figure 1. Mercury uptake by *Synedra* under various conditions.

Dividing (open circles), non-dividing (triangles) and heat-treated cells (closed circles) were incubated in a medium with 8 ppb $HgCl_2$ which was added at zero time. Dividing cells were incubated under illumination, while non-dividing cells were incubated in the dark. After the incubation the cells were collected on a glass fibre filter, followed by washing with medium (solid lines) or 0.1mM, pH 7.0 cysteine solution (dotted lines).

As to the total uptake which was determined after washing the cells with culture medium, the following observations were made. During the first few hours, both dividing and non-dividing cells took up mercury linearly with time. Since the amounts of mercury taken up into dividing cells were almost equal to that into non-dividing cells, this uptake may have resulted from passive adsorption rather than active uptake, as has been suggested by many reports. After this period, the uptake into non-dividing cells ceased, indicating that physical adsorption reached a saturation levels. On the other hand, the uptake into dividing cells was found to continue. This result strongly suggested that the process of the active uptake was working in the dividing cells, which played an important role in the accumulation of mercury. The accumulation of mercury into heat-treated cells was also found, but it probably resulted only from passive adsorption, because the cells were no longer alive. It is known that the influx of an external ion such as mercury into living cells is minimized by a relatively impermeable membrane and controlled by the specific exchange in many cases. Accordingly, the disruption of membranes should increse the permeability of cells to mercury resulting in an increment of the total uptake. This was clearly shown by the fact that heat-treated cells accumulated more mercury than non-dividing cells did.

As to the uptake into the cells, which was determined after washing the cells with a cysteine solution to eliminate mercury adsorbed physically to the surface, the following results were obtained. About 30% of mercury taken up by dividing cells could be removed by washing with a cysteine solution, indicating that at least this portion of the total uptake was due to the passive adsorption on the cell surface; the residual portion was considered to consist of mercury bound more tightly and /or accumulated in the cells. From this result it was obvious that total mercury taken up in cells was in two states, *i.e.*, one easily eluted by washing with a cysteine solution, and the other bound rather tightly. Mercury taken up by non-dividing cells was also in two states but the portion which was easily washed off with a cysteine solution was greater compared with dividing cells. On the other hand, although heat-treated cells took up more mercury than living cells, a large part of it was eliminated by washing with a cysteine solution and the amount of residual mercury became less than that of the dividing cells and equal to that of non-dividing cells. This fact indicated that an increased uptake by heat-treated cells was due to the disruption of membrane as discussed above. The nature of this residual portion is not clear at present.

However, since the amount of mercury which remained after washing with a cysteine solution was found to be much larger in dividing cells than in non-dividing cells, this portion of mercury, at least in dividing cells, might reflect the presence of the active uptake in these cells.

Effect of various ions on mercury uptake

Synedra showed little uptake of mercury in the standard buffer without addition of any ions, while it accumulated mercury to some extent in the buffer containing CO_3^{--} and NO_3^- at the same concentrations as in the culture medium, though to a lesser extent than that found in the culture medium. This fact indicated that cells required some other components than these ions for the mercury uptake. Thus, the effect of various ions on the uptake by *Synedra* was investigated and the results are presented in TABLE 1. A much greater uptake of mercury

TABLE 1.

Requirements for mercury uptake by *Synedra*

Exp.	Standard buffer	Addition to standard buffer (mM) Mg^{++} (o.16)	Ca^{++} (o.48)	SiO_3^{--} (o.16)	Fe^{+++} (o.oo6)	Radioactivity taken up (cpm)
1	+					146
2	+	+				368
3	+		+			138
4	+			+		153
5	+				+	68
6*	+	+	+	+	+	315

Mercury accumulated in *Synedra* was measured after a 24 hours incubation in culture medium or in standard buffer supplemented with various ions as described in text.
* CHU No.16 medium.

was observed in a standard buffer supplemented with Mg^{++} than in the buffer without Mg^{++}, and the level of uptake reached to the one obtained in the culture medium (CHU No. 16 medium). On the other hand, the presence of ions other than Mg^{++} which were usually added as nutrient ions had no effect on the uptake of mercury; even some suppressive effects were observed in the case of Fe^{+++}.

The effect of Mg^{++} on the mercury uptake was studied in detail as shown in Figure 2. It is interesting to note that the uptake increased linearly with time in a standard buffer with Mg^{++}, while in the buffer without Mg^{++} it increased for a first few hours, but became much more sluggish and finally stopped. However, when Mg^{++} was added to the culture at this point, the uptake was again restored as shown in Figure 3, although its velocity was slightly slower than that of first stage.

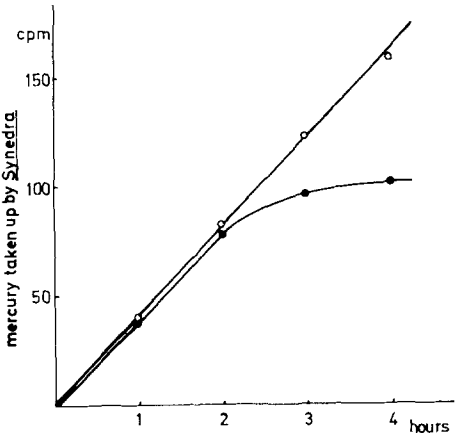

Figure 2. Effect of Mg^{++} on mercury uptake by *Synedra*

^{203}Hg was added at zero time to the suspension of *Synedra* in a standard buffer either with (open circles) or without 0.1mM Mg^{++} (closed circles).

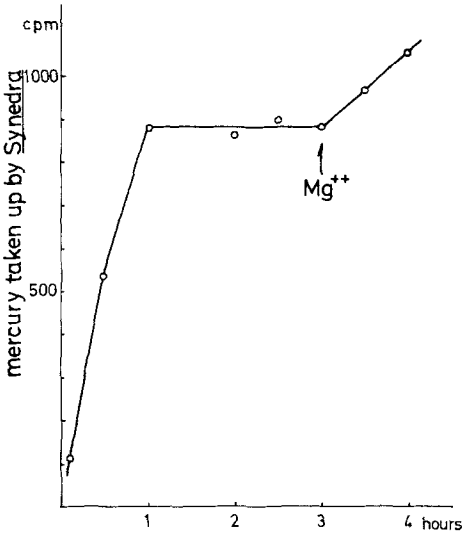

Figure 3. Effect of Mg^{++} on mercury uptake by *Synedra*

Incubation was carried out in a standard buffer as described in text. ^{203}Hg was added at zero time. 0.1 mM Mg^{++} was further added at the point indicated.

This result indicated that Mg^{++} was absolutely required for the uptake of mercury by planktons. Figure 4 shows the effect of the concentration of Mg^{++} on the uptake of mercury. From the figure it is clear that an optimal concentration of Mg^{++} ion for the uptake was about 0.1mM.

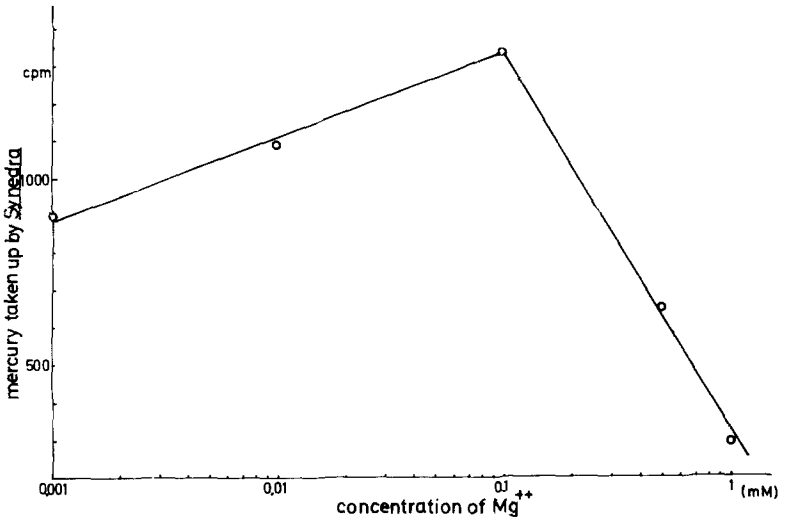

Figure 4. Mercury uptake in *Synedra* as a function of Mg^{++} concentration.

Incubation was carried out in a standard buffer with varying concentration of Mg^{++}. Mercury uptake was measured at 24 hours after ^{203}Hg addition.

Effect of inhibitors

The fact that the mercury uptake was stimulated by light together with Mg^{++} as described above suggested that energy-linked metabolism might be responsible for the enhanced uptake of mercury. As shown in TABLE 2, cyanide and azide, which were known to be potent inhibitors of respiratory enzymes, effectively prevented the mercury uptake. In the presence of 0.01mM CN^- the uptake was reduced to about 50% of the control. Azide also inhibited the uptake, but less efficiently.

The effect of CN^- on the uptake of mercury was examined using cells suspended in culture medium with or without 0.01mM CN^-. A considerable depression of the mercury uptake was observed with the cells suspended in the culture containing CN^- for the first few hours after an addition of mercury. However, the depression was significantly released after 24 hours and the uptake was gradually increased thereafter (Figure 5). This might be due to the increase of the passive adsorption to dead cells, since it was shown that the cells grown

TABLE 2

Effect of inhibitors on mercury uptake by *Synedra*

Concentration of inhibitors (mM)	Mercury taken up in *Synedra* cells cpm	%
None	4451	100
N_3^- (0.1)	3784	85.0
CN^- (0.01)	2289	51.4
// (0.05)	2132	47.9
// (0.1)	1950	43.8

After preincubation for 30 minutes in the medium with inhibitors. ^{203}Hg was added to the medium at 8 ppb and incubation was continued for 24 hours. The cells were filtered, washed with 0.1mM cysteine solution (pH 6.5) and the radioactivities were measured.

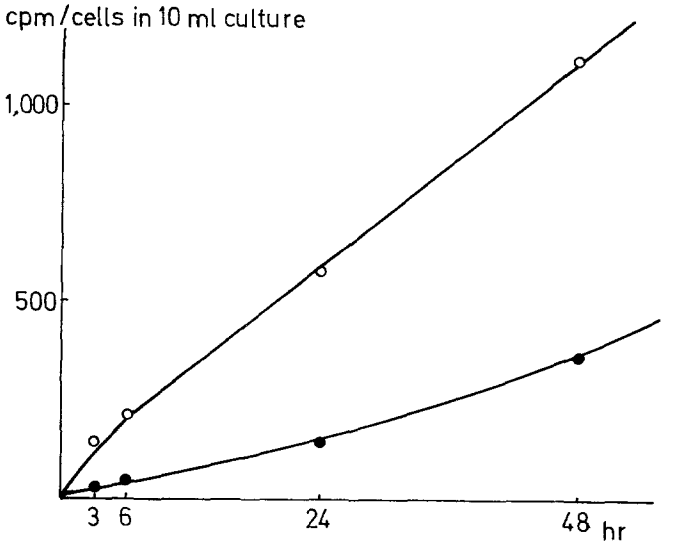

Figure 5. Effect of CN^- on mercury uptake by *Synedra*

Incubation was carried out in a culture medium either with 0.01mM CN (closed circles) and without it (open circles). ^{203}Hg was added at zero time.

in the culture with CN^- for 48 hours were not able to divide. The fact that CN^- depressed the uptake of mercury by planktons suggested that the mercury was taken up through a pathway linked to energy metabolism. From this aspect it is interesting to note the reports by the several authors that the divalent ions such as calcium, strontium, manganese, barium and magnesium are accumulated in mitochondria and that the uptakes of these cations are linked to the electron transport in mitochondria, which requires respiratory substances as well as Mg^{++} (LEHNINGER et al, 1967, RASMUSSEN and OGATA, 1966). It has been

also reported by WALTON (1973) that the accumulation of lead in mitochondria was found to occur by a process dependent on energy. Thus, it is very interesting to investigate the process of the active uptake of mercury in *Synedra* in relation to the energy metabolism.

Conclusion

The uptake of mercury by *Synedra* was investigated under several conditions. It was found that the uptake was much higher under illumination than in the dark. This increase was suggested the presence of an active uptake linked to the energy metabolism, which required light and Mg^{++}. It was also shown that the uptake observed in a dark or by dead cells was due to passive adsorption.

Acknowledgment

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