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Changes in activity of certain enzymes in sea urchin embryos and larvae after exposure of adult organisms to heavy metals

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ABSTRACT: The activity of acid and alkaline phosphatases, glucose-6-phosphatase, Mg^{2+}-ATPase and glucose-6-phosphate dehydrogenase was investigated in embryos and larvae obtained from gametes of the sea urchin *Strongylocentrotus nudus* exposed for 30 d to increased zinc, copper and cadmium concentrations (100, 25 and 50 µg l^{-1}, respectively). Exposure of parent sea urchins to zinc resulted in increased Mg^{2+}-ATPase activity and decreased glucose-6-phosphate degydrogenase activity in larvae at the stage of 2 d pluteus, followed by their death. Exposure of parents to copper resulted in accelerated development of larvae at the stage of early pluteus; exposure to cadmium led to no visible changes in larval development.

INTRODUCTION

Extended exposure of marine invertebrates to high concentrations of heavy metals causes an increase in the number of anomalous and non-viable embryos and larvae (Zaroogian & Morrison 1981, Khristoforova et al. 1984). Deviations from normal development may result from changes in enzymic activities (Evola-Maltese 1957).

To elucidate the mechanism of heavy metals' effect on the offspring, a laboratory experiment was carried out with a long exposure of the sea urchin *Strongylocentrotus nudus* to increased concentrations of zinc, copper and cadmium, followed by determination of metal accumulation in eggs and measurement of activity of acid and alkaline phosphatases, glucose-6-phosphatase, Mg^{2+}-ATPase and glucose-6-phosphate degydrogenase in the early stages of embryonic and larval development.

MATERIAL AND METHODS

The research was performed at the 'Vityaz' marine experimental base of the Far Eastern Branch of the USSR Academy of Sciences in June to August 1984. Four groups of adult sea urchins *Strongylocentrotus nudus* (each consisting of 35 individuals) were maintained in 100 l aquaria of plastic glass with aerated seawater. The water was changed every 2 d. One group was maintained in natural seawater (control). In the other 3 aquaria, after a regular water change, copper chloride, zinc chloride or cadmium chloride was added (25 µg Cu^{2+} l^{-1}, 100 µg Zn^{2+} l^{-1} and 50 µg Cd^{2+} l^{-1}, respectively). The aquaria were maintained for 30 d. Sea urchins were fed on sea algae, *Laminaria* and *Ulva*. Each portion of algae was utilized for 2 d. After 16 d exposure to copper, the sea urchins no longer consumed all the food offered.

At the end of the experiments, sex cells were obtained from 3 females and 3 males of each group. Within each group the eggs were pooled and used for subsequent work. After artificial fertilization of a small number of eggs the development of embryos of control and experimental groups was followed in 3 parallel experiments.

In the first experiment, embryos were cultivated in filtered seawater without additions of toxicants at 21 °C in 0.2 l beakers. At the zygote stages, 2 blastomeres, blastula and gastrula were selected, fixed with 0.2 % formalin diluted with seawater and for each 100 embryos the number of normally developing specimens was calculated. Using the method of Naidenko (1983), the cultivation of remaining control and experimental larvae was continued at 21 to 23.5 °C for 1 mo until first juvenile sea urchins appeared.

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The largest proportion of eggs was placed into 2 l glasses, fertilized and cultivated at constant stirring. At the stage of zygote, blastula, gastrula and 2 d pluteus, the activity of acid and alkaline phosphatases was determined (Sullivan & Volcani 1974), as well as that of Mg^{2+}-ATPase (Stewart 1974) and glucose-6-phosphatase (Barber & Foy 1973); at the stages of gastrula and 2 d pluteus, the activity of glucose-6-phosphate dehydrogenase was determined (Kochetov 1980). Suspensions of embryos in 0.05 M Tris-buffer (pH 7.5) were treated 3 times on an ultrasonic disintegrator UZDN-1 at 44 kHz for 10 s to destroy cellular membranes and centrifuged at 16000 × *g* for 20 min. The supernatant was treated as an enzymic preparation and its protein content determined as described by Lowry et al. (1951).

The remaining part of the eggs from the control and experiments was dried at 85°C to constant weight. After acid mineralization, copper, zinc and cadmium content was measured using a Shimadzu AA-610 S flame atomic absorption spectrophotometer.

RESULTS

Metal contents in eggs from control and experimental groups of sea urchins are given in Table 1.

Table 1. *Strongylocentrotus nudus*. Zinc, copper and cadmium contents in eggs of sea urchins (μg g^{-1} dry mass) after 30 d exposure to increased concentrations of these metals in water. Means ± SD; n = 3

Toxicant		Content in eggs Zn	Cu	Cd
Control		3.6 ± 0.2	0.03 ± 0.01	0.03 ± 0.01
Zinc	100 μg l^{-1}	6.2 ± 0.1••	0.08 ± 0.03	0.01 ± 0.01
Copper	25 μg l^{-1}	2.8 ± 0.1•	0.10 ± 0.03	0.03 ± 0.01
Cadmium	50 μg l^{-1}	3.6 ± 0.1	0.05 ± 0.01	0.03 ± 0.01

• Significantly different from control at $p < 0.05$
•• Significantly different from control at $p < 0.01$

In Zn-exposed specimens an accumulation of the metal in eggs was observed. In experiments with Cu and Cd, no accumulation in sex cells was observed.

The embryonic development of the offspring obtained from Zn- and Cd-exposed individuals was distinct from that in the control group (Table 2). Thus, 5 min after artifical fertilization the percentage of fertilized eggs was highest in the experiment with Cd and lowest in that with Zn. However, 1 h 10 min after fertilization, the amount of unfertilized eggs was less than 10% in all groups except Zn-exposed individuals. In the offspring of Zn-exposed specimens, half of the eggs remained unfertilized. The percentage of normally developing blastulas obtained from Cd-exposed sea urchins was lower compared to the control. In gastrulas, the development of embryos was similar in all groups.

The development of offspring obtained from gametes of Zn-exposed sea urchins was unsuccessful: the larvae died at the pluteus stage with 2 pairs of arms (Fig. 1c). In the offspring obtained from gametes of Cu-exposed sea urchins, the third pair of arms was formed faster than in the control (Fig. 1a, b). Plutei obtained from gametes of Cd-exposed individuals (Fig. 1d) were similar to the control ones.

On the 31st to 33rd days of development in cultures of plutei obtained from gametes of control sea urchins and those exposed to Cu and Cd, the first juveniles were found.

Results for enzyme activities in embryos and larvae are given in Table 3.

The activity of acid phosphatase in control embryos did not change during the process of development. In the offspring of Zn-exposed specimens, a rise in the acid phosphatase activity occurred in zygote and blastula; in gastrulas, a decrease in this enzyme activity was observed, and in plutei, it was restored to the control value. In embryos of Cu-exposed sea urchins, the activity of the enzyme was increased at all stages. In the offspring of Cd-exposed individuals, an increase

Table 2. *Strongylocentrotus nudus*. Embryonic development of sea urchin offspring after 30 d exposure of adult specimens to increased zinc, copper and cadmium concentration in water. Values are percentages of individuals in the stage indicated which survived the previous stage; means ± SD; n = 3

Age	Stage of development	Conditions of maintenance of adults Control	Zn	Cu	Cd
5 min	Zygote	50.0 ± 8.7	38.3 ± 6.1	59.6 ± 13.7	83.0 ± 4.6•
1 h 10 min	2 blastomeres	75.0 ± 2.1	35.6 ± 9.0•	70.6 ± 2.7	84.0 ± 5.1
	Zygote	22.3 ± 2.0	13.0 ± 4.2	19.3 ± 1.7	7.3 ± 1.5•
	Unfertilized eggs	1.3 ± 1.3	50.3 ± 13.3•	4.6 ± 1.5	8.3 ± 3.9
7 h 20 min	Blastula	84.1 ± 2.7	80.0 ± 1.1	78.0 ± 7.8	68.3 ± 3.1•
20 h 30 min	Gastrula	81.0 ± 7.8	79.1 ± 6.5	85.0 ± 1.0	74.3 ± 8.7

• Significantly different from control at $p < 0.05$

of activity of the enzyme was observed at all stages with the exception of blastula.

The activity of alkaline phosphatase in control embryos and larvae increased during development. In the offspring of Zn-exposed sea urchins, a rise in activity was observed only in blastulas. In the offspring of Cu- and Cd-exposed individuals, the activity of alkaline phosphatase increased in the plutei as compared with the control.

The activity of glucose-6-phosphatase in control embryos and larvae was low. In experimental groups, the activity of this enzyme remained at the control level except for gastrula where it was not found.

The activity of Mg^{2+}-ATPase in control larvae decreased during development. In the offspring of Cu- and Cd-exposed sea urchins the activity of the enzyme did not differ reliably from the control. Exposure of sea urchins to an increased Zn concentration resulted in an inhibition of Mg^{2+}-ATPase in gastrula and a sharp rise of activity in pluteus.

The activity of glucose-6-phosphate degydrogenase in control larvae did not practically differ in gastrula and pluteus stages. After Cu exposure, a decrease of enzymic activity was observed only in gastrulas, but it returned to control levels in plutei. After Cd exposure the activity of glucose-6-phosphate dehydrogenase in gastrulas was at the control level, but it increased in the pluteus stage. In larvae obtained from gametes of Zn-exposed sea urchins, the activity of the enzyme was reduced.

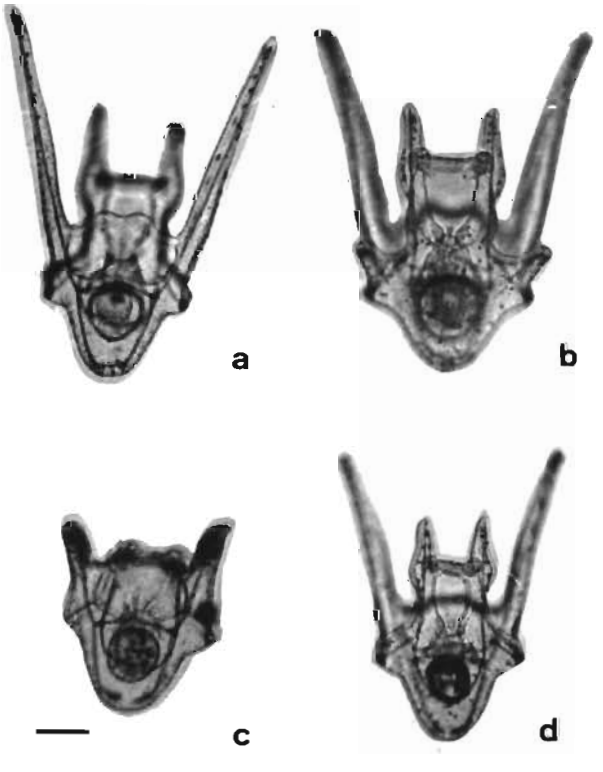

Fig. 1. *Strongylocentrotus nudus*. Plutei at age 9 d obtained from gametes of (a) controls, (b) copper-exposed, (c) zinc-exposed, (d) cadmium-exposed adults. Bar = 100 µm

DISCUSSION

These experiments show that long-term exposure of sea urchins to zinc, as distinct from copper and cadmium, resulted in bioaccumulation of this metal in eggs. A decrease of the percentage of fertilized eggs seemed to be a manifestation of the toxicity effect of zinc on the offspring. The same picture was observed in the fish *Brachydanio rerio* after exposure to 5 mg l^{-1} Zn for 9 d (Speranza et al. 1977).

In larvae obtained from gametes of Zn-exposed sea urchins, the activity of glucose-6-phosphate dehydrogenase and Mg^{2+}-ATPase changed.

Glucose-6-phosphate dehydrogenase is a key enzyme for transformation of carbohydrates through the pentose phosphate cycle, the product of which is riboso-5 phosphate, an RNA structural component. In sea urchins this route of carbohydrate transformation predominates in the early developmental stages (Giudice 1973). The activity of glucose-6-phosphate dehydrogenase in embryos increases immediately after fertilization, continues increasing up to the start of gastrulation and then falls gradually (Backström 1959). In our experiments the enzyme activity in the control offspring in gastrula and pluteus stages was practically the same. In the offspring of Zn-exposed specimens the enzyme activity was sharply decreased compared with the control. It was previously shown that treatment of sea urchin embryos with zinc sulphate solution resulted in a strong inhibition of synthesis of ribosomal RNA (Pirrone et al. 1970). Death of larvae obtained from gametes of Zn-exposed parents seems to be connected with an inability to build their own protein-synthesizing apparatus, because in the period from mesenchymatous blastula to pluteus (age 70 to 80 h), a gradual substitution of ribosomes produced in oogenesis by those synthesized by the embryo itself occurs (Nemer & Infante 1967).

The activity of Mg^{2+}-ATPase in the control offspring in gastrula was twice as high as that in plutei. Exposure of adults to Zn resulted in a reduction of the enzyme activity in gastrula and, in contrast, in its almost 3-fold increase in plutei. From literature data we known that Zn can both raise and reduce the activity of Mg^{2+}-ATPase, which seems to be dependent on metal concentration and peculiarities of the studied tissues. Thus, in the rainbow trout *Salmo gairdneri*, the activity of Mg^{2+}-ATPase in gills increased at Zn concentration 0.29 mg l^{-1} but remained constant at 0.99 and 1.98 mg l^{-1} (Watson & Beamish 1980). In the crustacean

Table 3. *Stronglocentrotus nudus*. Activity of enzymes in the offspring of sea urchin after 30 d exposure of adults to increased zinc, copper and cadmium concentrations. Means ± SD; n = 4

Conditions of maintenance	Developmental stages	Acid phosphatase	Alkaline phosphatase	Glucose-6-phophatase	Mg^{2+}-ATPase	Glucose-6-phosphate dehydrogenase
		(μg P inorg. h^{-1} mg^{-1} protein)				(nmole min^{-1} mg^{-1} protein)
Control	Zygote	37.8 ± 1.0	6.4 ± 0.6	7.0 ± 1.6	94.7 ± 5.0	–
	Blastula	33.1 ± 2.6	6.2 ± 0.5	7.6 ± 0.4	96.6 ± 5.8	–
	Gastrula	36.9 ± 1.0	7.1 ± 0.9	2.0 ± 0.5	79.3 ± 9.2	39.0 ± 6.6
	Pluteus	41.3 ± 3.0	8.5 ± 0.1	1.6 ± 0.4	39.3 ± 5.0	38.7 ± 0.6
Zinc 100 μg l^{-1}	Zygote	46.2 ± 1.6••	7.2 ± 0.5	10.5 ± 0.3	108.0 ± 7.2	–
	Blastula	46.8 ± 2.0•	8.7 ± 0.5•	9.3 ± 0.7	100.6 ± 11.5	–
	Gastrula	23.3 ± 2.1••	6.6 ± 0.1	nd	31.3 ± 8.1 •	8.7 ± 2.4••
	Pluteus	40.9 ± 1.6	9.9 ± 0.7	1.2 ± 0.0	86.6 ± 11.5•	12.2 ± 2.2••
Copper 25 μg l^{-1}	Zygote	53.4 ± 1.6••	6.5 ± 0.6	11.4 ± 0.6	103.3 ± 5.7	–
	Blastula	55.4 ± 3.0••	6.5 ± 0.8	11.6 ± 0.7••	101.3 ± 2.3	–
	Gastrula	50.5 ± 0.2••	8.2 ± 0.9	nd	70.0 ± 10.0	32.3 ± 1.5•
	Pluteus	64.3 ± 0.5••	11.8 ± 0.8•	1.9 ± 0.2	55.0 ± 3.2	47.5 ± 5.0
Cadmium 50 μg l^{-1}	Zygote	51.1 ± 2.2••	7.0 ± 0.3	11.8 ± 0.3•	90.0 ± 17.3	–
	Blastula	44.0 ± 3.2	7.3 ± 0.6	10.4 ± 0.3	98.0 ± 3.5	–
	Gastrula	42.7 ± 0.6••	7.4 ± 0.3	nd	63.3 ± 5.7	35.5 ± 5.2
	Pluteus	52.8 ± 2.0•	11.5 ± 0.7•	1.9 ± 0.2	49.0 ± 6.4	67.0 ± 9.2•

• Significantly different from control at $p < 0.05$
•• Significantly different from control at $p < 0.01$
nd: not detected
–: not measured

Homarus americanus, a Zn concentration of 25 mg l^{-1} inhibited the activity of gill ATPases; when the lobsters were placed into clean water the activity of enzymes was not restored (Haya et al. 1983).

The activity of glucose-6-phosphatase in the control offspring was at practically the same level in zygote and blastula and decreased sharply in gastrula and in pluteus. After exposure of adults to increased concentrations of heavy metals, the activity of this enzyme was not observed in the offspring in gastrula stage, whereas in pluteus it appeared again. it is well-known that most embryonic enzymes are already present in the egg. In the process of development, they are replaced by molecules synthesized by the embryo itself. For example, in sea urchins, after late gastrula stage, genetic control of aldolase and esterase synthesis was observed (Neifakh & Timofeeva 1978). It is possible that, in our case, restoration of glucose-6-phosphatase activity in the pluteus is connected with the effect of embryonic genome.

The exposure of sea urchins to increased metal concentrations resulted in a increase of acid phosphatase activity in embryos and larvae. Measurements of this lysosomal enzyme activity in the sea urchin *Strongylocentrotus purpuratus* inhabiting polluted environments (Jenkins et al. 1982), in the fish *Heteropneustes fossilis* (Sastry & Subhadra 1985) and in the bivalve *Protothaca staminea* (Roesijadi 1980) in experimental conditions show its tissue specificity and dependence on metal concentrations. In our experiments the increased activity of acid phosphatase seems to result from the stress effect of toxicants on the parents.

In conclusion, a long exposure of sea urchins to increased Zn concentrations resulted in inhibition of glucose-6-phosphate dehydrogenase and activation of Mg^{2+}-ATPase in larvae and their death. In the offspring of Cu-exposed individuals, an acceleration in development was observed in the stage of early pluteus. In the experiment with Cd, the change in activity of certain enzymes in embryos and larvae did not influence the development of the sea urchins.

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