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Isotope Ratios in Marine Mollusk Shells after Prolonged Contact with Flowing Fresh Water

Abstract. *The ratios C^{13}/C^{12} and O^{18}/O^{16} in the calcium carbonate of shells of the marine mollusk,* Macoma calcarea, *appear unaltered after exposure for 4500 years to flowing fresh water of higher O^{16}/O^{18} ratio.*

The widespread use of carbon and oxygen isotopes as paleoenvironmental indicators in sedimentary geochemistry (*1*), for radiocarbon dating, and for paleotemperature determinations (*2*), depends to a large extent on the assumption that the original isotopic composition has not been altered by postdepositional, diagenetic processes (*3*). While oxygen isotope exchange in the calcium carbonate–water system proceeds relatively rapidly at high temperatures (*4*), it appears that original carbon isotope ratios may be preserved in calcitic limestones over very long periods of time, as indicated by consistent changes in the values of δC^{13} found in a regional study of isotope ratios in the Vanport limestone of Pennsylvanian age (*5*). Furthermore, in a study of over 500 marine and fresh water carbonates of Phanerozoic age (*6*), the original carbon isotope ratios appear to have remained unchanged since the Devonian—the earliest fresh-water limestone known to us is Devonian—because the expected difference in mean isotopic composition of marine and fresh-water limestones was confirmed. These samples, however, were mostly compact, dense limestones which were apparently not subjected to a significant flow of intrastratal solutions around grain boundaries.

Table 1. Carbon and oxygen isotope ratios of six individual shells of the marine mollusk, *Macoma calcarea*. The values, per mill, are relative to Chicago PDB-standard carbon dioxide.

δC^{13}	δO^{18}
−1.56	+.72
−1.64	−.58
−1.77	+.40
−1.47	+.98
−1.51	+.58
−1.37	−.43
Mean	
−1.55	+.28
S.D.	
.141	.636

* Collected by J. F. Schwietering, from north bank of Rivière du Sud, 1.6 Rm east of St. François, Montmagny county, Quebec, now 45 m above sea level.

Gross (*7*) reports that marine Pleistocene limestone from Bermuda and from the atolls, Bikini and Eniwetok, has been altered by the Ghyben-Herzberg lens of fresh water and by the precipitation of secondary calcite in a relatively short period of time so that C^{13}/C^{12} and O^{18}/O^{16} ratios approach those of fresh-water limestones. Unpublished data from this laboratory suggest that, if certain corals have contributed calcium carbonate to the calcareous sediments, some of these anomalous isotope ratios may, in fact, be the original ratios which obtained at the time of deposition.

Because of the importance of the preservation of the original isotopic record in carbonates, a collection of marine shells of the mollusk *Macoma calcarea* which were exposed to percolating freshwater for a known period of time, was made and analyzed for isotope ratios by standard techniques (*8*). After deposition in the bottom sediments of the Champlain Sea between 9500 and 9900 years ago (*9*), the mollusk shells have been elevated and subjected to the effects of percolating fresh water in the banks of Rivière du Sud, Quebec, for at least 4500 years. The isotopic composition, expressed as the difference in C^{13}/C^{12} or O^{18}/O^{16} ratio of the sample and the Chicago PDB standard carbon dioxide, in per mill, by the formula

$$\delta C^{13} = \left(\frac{C^{13}/C^{12}_{\text{sample}}}{C^{13}/C^{12}_{\text{std.}}} - 1 \right) 1000,$$

has been corrected for errors of measurement, such as capillary leaks and the presence of O^{17} (*10*), and is presented in Table 1. The results are well within the accepted range of marine carbonates (*11*), and indicate that under some conditions original isotope ratios may be unaltered or altered to an indetectable extent, after contact with flowing fresh water for as long as 4500 years.

The statement of Rubin and Taylor (*3*), that the degree of alteration can be determined by mass spectrometric studies of isotopes is not necessarily true, especially in the case of fresh-water mollusk shells, some of which originally exhibit a wide range in isotopic composition (*12*; *13*).

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Zinc Activation of a Coordinated Response in *Hydra*

Abstract. *In* Hydra littoralis, *the feeding response normally activated by reduced glutathione can be elicited by zinc ions under special experimental conditions: some calcium is necessary for the activation to be realized, but a high concentration of calcium inhibits the zinc-activated response. Zinc inhibits the normal feeding response induced by reduced glutathione.*

Zinc ions are known to have immediate physiological effects at several levels of biological organization. Vallee *et al.* showed that the addition of zinc to the apoenzyme of carboxypeptidase activates that enzyme (*1*). Isaacson and Sandow found that zinc potentiates the twitch of muscle (*2*). In this report we describe the activation by zinc of the feeding response in *Hydra littoralis*.

The feeding response of *Hydra* is normally activated by the ubiquitous

tripeptide, reduced glutathione (*3*). In an attempt to determine the mechanism by which the glutathione-receptor is activated, we treated *H. littoralis* with non-tripeptides, such as enzymes and metals, that might behave either as activators or as inhibitors. Most heavy metals and metal derivatives, such as copper and *p*-mercuribenzoate, inhibit the feeding response to glutathione (*4*). Therefore, we were surprised to discover that zinc ions, in the absence of added glutathione, activated the feeding response. Furthermore, the same concentrations of zinc inhibited the response to reduced glutathione, the degree of inhibition depending upon the experimental conditions.

The feeding response was measured as previously described (*5*). Mass cultures of *H. littoralis* (*6*) were grown and maintained in a medium of $10^{-3}M$ $CaCl_2$ and $10^{-4}M$ $NaHCO_3$. They were kept without food for 2 days prior to use. During the experiments the *Hydra* were placed in a standard solution consisting of $10^{-3}M$ histidine buffer, *p*H 5.9, and $10^{-4}M$ NaCl, to which various other substances were added.

When *H. littoralis* were treated with standard solution to which both $10^{-3}M$ $CaCl_2$ and $10^{-4}M$ $ZnCl_2$ had been added, they writhed their tentacles slightly, but no feeding response was observed (Table 1, expt. 1*A*). When others were placed in the same solution containing $10^{-5}M$ glutathione they responded for 28.5 minutes (expt. 1*B*), which was a somewhat lower response than that given by the control group responding to glutathione without added zinc (expt. 1*C*). This inhibition was slight compared with that given by equal concentrations of copper under the same conditions (*4*).

The conditions leading to activation by zinc (Table 2, expt. 2*A*) differed from those in experiments 1*A* and 1*B* in that neither calcium nor glutathione had been added to the standard solution. A fresh group of *H. littoralis* was rinsed for 1 minute in the standard solution, and then placed in the standard solution to which $10^{-4}M$ $ZnCl_2$ had been added. These animals responded for an average of 9 minutes (expt. 2*A*); one or two of them occasionally responded for as long as 15 minutes. This same group of *Hydra* was then washed three times with the standard solution containing $10^{-3}M$ $CaCl_2$, and was then left in this solution for 15 minutes so that the free and loosely bound zinc would be removed and to allow free calcium to reassociate with the *Hydra* (*7*). The same animals were then placed in the standard solution to which $10^{-3}M$ $CaCl_2$ and $10^{-5}M$ glutathione had been added; the animals did not respond (expt. 2*B*). Presumably, some zinc was still bound to the *Hydra* and interfered with the activation by glutathione. Therefore, in order to allow more of the zinc to dissociate, groups of *Hydra* treated as in experiment 2*A* were maintained in the standard solution containing $10^{-3}M$ $CaCl_2$ for varying periods. After 4 hours the *Hydra* responded for 9.3 minutes to glutathione (expt. 2*C*), and after 18 hours, they gave a significant response (expt. 2*D*). Still another group of *H. littoralis* was kept in the calcium solution for 2 days; there were no signs of inhibition since these *Hydra* responded as long as did the controls.

Table 1. Zinc activation of a feeding response in *Hydra littoralis*. In each experiment the standard solution consisted of $10^{-3}M$ histidine buffer, *p*H 5.9, and $10^{-4}M$ NaCl.

Treatment	Experiment 1		
	A	*B*	*C*
Standard solution	+	+	+
$10^{-4}M$ $ZnCl_2$	+	+	−
$10^{-3}M$ $CaCl_2$	+	+	+
$10^{-5}M$ reduced glutathione	−	+	+
Duration of response* (min)	0	28.5	35.3

* Expressed as the mean response for five animals tested.

Previous experiments showed that calcium ions were required for the response activated by glutathione (*8*). Since zinc activated a response only when calcium was omitted from the medium (expt. 2*A*), we added zinc to *H. littoralis* that had been in the calcium-free standard solution for periods of 1 to 40 minutes (*8*). Zinc did not activate the response either in the presence of calcium ions (Table 3, expt. 3*A*), or after the animals had been kept in a solution lacking added calcium for 40 minutes (expt. 3*E*), but only when they had been without added calcium from 1 to 5 minutes (expts. 3*B* and 3*C*). Thus, as for the response activated by glutathione, some calcium ions are required for activation by zinc.

These results suggest that for zinc to activate, a delicate balance must exist between calcium and zinc; this balance could be upset by varying the concentration of either ion. For example, the response to zinc (under conditions of experiment 2*A*) could be interrupted at any time and stopped within 30 seconds by increasing the calcium concentration to $10^{-3}M$. On the other hand, when the zinc concentration was in-

Table 2. Zinc activation of the feeding response, and inhibition of the glutathione-activated response. The animals used in experiments 2*B*, 2*C*, and 2*D* were previously treated as in experiment 2*A*.

Treatment	Experiment 2			
	A	*B*	*C*	*D*
Incubated in:				
Standard solution (1 min)	+			
Standard solution + $CaCl_2$ (15 min)		+		
Standard solution + $CaCl_2$ (4 hr)			+	
Standard solution + $CaCl_2$ (18 hr)				+
Followed by standard solution containing:				
$10^{-4}M$ $ZnCl_2$	+	−	−	−
$10^{-3}M$ $CaCl_2$	−	+	+	+
$10^{-5}M$ reduced glutathione	−	+	+	+
Duration of response (min)	9.1	0.8*	9.3	22.6

* One animal gave a short response.

Table 3. Effect of incubation in calcium-free medium on activation by zinc.

Treatment	Experiment 3				
	A	*B*	*C*	*D*	*E*
No prior incubation	+				
Incubation in standard solution for:					
1 min		+			
5 min			+		
20 min				+	
40 min					+
Followed by:					
Standard solution + $10^{-4}M$ $ZnCl_2$	+	+	+	+	+
Duration of response (min)	0	8.8	2.0	0.6	0

creased to $10^{-3}M$ while the calcium was held at $10^{-3}M$, the animals responded for 4 minutes (*9*).

The feeding response elicited by zinc resembled the response activated by glutathione, although occasionally some tentacles assumed a transitory (2 to 3 seconds) hypercontracted appearance. Long exposure (18 hours) to $10^{-3}M$ zinc in the presence of $10^{-3}M$ $CaCl_2$ in the test solution proved toxic to *Hydra*. The zinc-activated response was an effective one in that the *Hydra* ingested small inert objects offered to them. Zinc also activated a response in *Hydra pirardi*.

To determine whether the activation by zinc was a typical response to metals, we tested the effects of copper, nickel, cobalt, cadmium, lead, and uranyl ions. None acted in the same way as zinc. On the contrary, depending upon the experimental conditions, these metals inhibited the feeding response to glutathione, and were toxic to *Hydra littoralis*.

Zinc-calcium interactions are known, for example, both in nutrition, where effects of high calcium diets can be relieved by zinc (*10*), and in muscle physiology, where the twitch potentiation of the frog's sartorius induced by zinc (*2*) can be reversed by calcium-ethylenediaminetetraacetic acid (*11*). In our experiments, calcium might either be competing with zinc for a single site on the receptor-effector system or be preventing zinc from reaching the site of its activity.

Since some of the biological and chemical actions of zinc and other non-tripeptide activators of the feeding reflex (*12*) are known, studies of their effects on *Hydra* might help elucidate the nature of the receptor or of some of the components of the receptor-effector system. For example, Gurd and Goodman (*13*) and Vallee *et al.* (*14*) have shown that zinc can be bound to the histidine, sulfhydryl, or terminal α-amino of proteins. Since studies of the effect of *p*H (*15*) indicate that histidine and an α-amino might be at the glutathione-receptor site, zinc could possibly activate feeding in *Hydra* by binding these groups.

The activation of feeding in nature by free zinc is probably not of ecological significance because a concentration as high as $10^{-3}M$ is toxic and would most likely not be encountered there. It is possible, however, that glutathione acts by making "bound" zinc available to a zinc-requiring apoenzyme. Alternatively, zinc, known to act on muscle (*2*), might cause effector myofibrils to contract. A third possibility is that zinc becomes bound to some of the receptors, causing a slight change in their tertiary structure in a manner analogous to that proposed for glutathione (*2*), thus leading to the activation of the receptor-effector system (*16*).

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Toxohormone Inhibitory Effect on the Growth of an Unstable Strain of Yeast

Abstract. *Toxohormone preparations obtained from yeast mutants with impaired respiration and from cancerous tissues inhibited the growth of an unstable strain of yeast in media containing lactate as the carbon source. Depending on the source of the different toxohormone preparations, amounts ranging from 5 to 9.5 mg/ml inhibited growth by 50 percent. This phenomenon could be utilized in quantitative evaluations of toxohormone activity.*

Unstable strains of yeast produce spontaneously large numbers of respiration-deficient (RD) mutants. In ordinary sugar media, populations of these unstable strains often contain more than 50 percent of RD mutants (*1*). When one such unstable strain was cultured in a medium containing lactate as the carbon source, it grew normally, but the number of living cells decreased rapidly after the 3rd day of inoculation so that the culture was almost sterile after 2 weeks.

Since respiration-deficient mutants

Table 1. Growth of an unstable strain of yeast (R6U2) in a medium containing lactate as the carbon source, to which toxohormone preparations were added at different concentrations. The results are expressed as millions of cells per milliliter after incubation for 24 hours. Each figure represents the mean of five experiments.

Source of toxohormone	Concentration of toxohormone (mg/ml)							
	30	25	20	15	10	5	1	0
T_7 RD yeast mutant	1.3	1.6	2.1	2.8	4.6	6.9	10.0	10.2
Mn6 RD yeast mutant	0.7	1.0	1.5	2.5	4.0	6.2	9.3	10.2
r 6u2 RD yeast mutant	1.1	1.7	2.4	3.4	4.9	7.3	9.5	10.2
S_2 normal yeast strain	9.9	9.5	11.0	11.3	10.8	10.0	10.6	10.2
Mammary carcinoma	1.0	0.8	1.2	1.4	2.5	5.0	8.6	10.2
Parotid gland carcinoma	0.8	0.8	1.0	1.9	3.3	5.7	9.1	10.2
Noncancerous muscular tissue	9.9	10.0	10.0	12.1	10.6	11.4	10.2	10.2