The Binding of Organic Mercurials by Normal and Sucrose-Swollen Embryos of the Sea Urchin *Paracentrotus lividus*

Living embryos of sea urchins take up increasing amounts of organic mercurials as they develop[1]. This observation suggests the continual appearance of new sulfhydryl groups at the surface of the embryos. It seems plausible that some of the mercurial is bound by materials holding the embryonic cells together. We have tried stretching the embryos by means of sucrose, as described by MOORE[2], to determine whether the osmotic swelling which results might influence the amounts of mercurials bound by the embryos.

Methods. Fertilized eggs of *Paracentrotus lividus* were reared in sucrose in sea water (1 part molar sucrose to 9 parts sea water) from two-cell stage until hatching was completed. Controls were similarly exposed to sea water diluted with a corresponding amount of isosmotic NaCl. After hatching, the embryos were removed to normal sea water where within 30 min they showed a marked increase in the diameter of the blastocoel and a thinning of the blastocoel wall. Control embryos did not show such changes.

The extent of binding of organic mercurials by swollen and control embryos was determined by exposing 300,000 embryos to a measured amount of mercurial for 3 h, and then assaying the amount of mercurial left in the supernatant after removal of the embryos, according to the method of FRIDOVICH and HANDLER[3]. 75 mμmoles of paramercurichlorobenzoate (PCMB) or parachloromercuribenzene sulfonate (PCMBS) were added to 2–4 ml sea water and 0.5*M* NaCl solution (1:1) containing 300,000 sucrose-treated or control embryos, and left to stand at room temperature for 3 h. In a parallel series of experiments the mercurial was added at the end of the 3 h incubation to measure any mercury-binding material released by the embryos during the incubation period. At the end of the incubation the embryos were separated by centrifugation and the supernatant was assayed for remaining mercurial by the addition of phosphate buffer, pH 7, to a concentration of 0.1*M*, ethylene diamine tetracetate (EDTA) to 0.01*M*, followed by dithizone in carbon tetrachloride adjusted to an absorbance of 0.85 O.D. After 1 min of shaking the cold mixture, the absorbance of the CCl_4 phase was read at 625 nm. This absorbance is directly proportional to the amount of mercurial bound by the embryos.

Results. Embryos reared in sucrose and removed after hatching to normal sea water showed a typical swelling of the blastocoel with thinning of the blastula wall. They developed at the same rate as controls both before and after hatching, but the onset of hatching and its completion were slightly delayed in the treated embryos.

Amounts of organic mercurials bound by 300,000 normal and sucrose-swollen embryos of *P. lividus* after 3 h exposure

Experiment	Reagent	Normal (mμmoles mercurial)	Sucrose-swollen (mμmoles mercurial)
1	PCMB	32	32
2		40	36
3	PCMBS	12	12
4		11	8

The sucrose-treated embryos bound PCMB and PCMBS to the same extent as did the normal embryos. (Table).

Both treated and control embryos bound about 35 mμmoles of PCMB, and about 10 mμmoles of PCMBS. The PCMBS is thought to penetrate charged diffusion barriers less readily than PCMB and sea urchins have previously been shown to bind PCMBS less than PCMB at various stages of development[1]. Our present results suggest that neither the increase in size of the blastocoel nor the concomitant thinning of the blastocoel wall involves a change in the number of sulfhydryl groups, measurable with the present assay, at or near the surface of the blastomeres.

A difference in behavior during exposure to the mercurials was observed in the treated and control embryos. Normal embryos exposed to the organic mercurials swam more slowly than unexposed embryos, whereas swollen embryos swam at their original rate even in the presence of mercurials. Since ciliates are immobilized by and bind these same mercurials the effect on ciliary activity may be a general one[4]. However, the difference in the swimming activity of normal and swollen embryos in the presence of mercurial suggests that the location of the mercurial binding sites may differ between the two.

Conclusions. The organic mercurials which attach to the living embryos during the period of assay are thought to react with free sulfhydryl groups at the surface of blastomeres. The number of these groups appears not to change in response to mechanical strain imposed on the cellular surfaces and junctions by the osmotic forces which swell the embryos reared in sucrose[5].

Résumé. En vue d'étudier les effets d'une tension intercellulaire sur les groupes sulfhydriles à la surface des blastomères, nous fîmes subir aux blastules d'oursin un gonflement osmotique, en les traitant au sucrose. Les embryons enflés absorbèrent ensuite autant de réactif mercuriel que les embryons normaux. Nous en déduisons que les groupes sulfhydriles superficiels ne sont pas influencés par les forces osmotiques qui déforment les parois des embryons.

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[1] N. WOLFSON, C. r. Séanc. Soc. Biol. *160*, 1996 (1966).
[2] A. R. MOORE, J. exp. Zool. *84*, 73 (1940).
[3] I. FRIDOVICH and P. HANDLER, Anal. Chem. *29*, 1219 (1957).
[4] S. F. BOWLES and N. WOLFSON, Proc. Can. Fedn. Biol. Soc. *10*, Abst. 224, p. 84 (1967).
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