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Embryotoxic and teratogenic effects of styrene derivatives on sea urchin development

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PAGANO, G., ESPOSITO, A., GIORDANO, G. G. and HAGSTRÖM, B. E. Embryotoxic and teratogenic effects of styrene derivatives on sea urchin development. *Scand. j. work environ. & health* 4 (1978): suppl. 2, 136—141. The effects of styrene and some of its derivatives on the fertilization and differentiation of sea urchins was investigated. When one of the gametes was pretreated for a few minutes, it was ascertained that the test substances act on the haploid nucleus, producing specific changes in the differentiation of the embryo. By this test system directly acting, weak mutagens may be detected.

Key words: development, fertilization, mutagenicity, styrene derivatives.

This report concerns the effects of styrene and some of its derivatives on the gametes and embryos of sea urchins. These organisms are highly organized, they have a high number of chromosomes (2n is ~ 36 in most species), and the early stages of embryonal differentiation closely resemble those of mammalian ontogenesis. Since sea urchins have been in the center of experimentation on fertilization, development and physiology for a century, the accumulated information is extensive (1, 3). In this context it should also be pointed out that the classical work on chromosomes was carried out by Boveri, who also used sea urchin embryos (1). Gametes and embryos have been used as a model test system in a number of investigations dealing with, for example, substances employed in pharmacology (3, 4), the effects of oil and oil dispersants (5), and carcinogenic polycyclic hydrocarbons (2).

Styrene oxide is a recognized mutagen (6, 7, 8, 9, 10). The mother substance of the various derivatives used in this study is styrene, and through the transformations depicted in fig. 1, the derivatives were obtained.

MATERIAL AND METHODS

The experiments were carried out at Stazione Zoologica, Naples, with eggs, sperm and embryos of two species, *Paracentrotus lividus* and *Psammechinus microtuberculatus,* in various stages of development.

The methods employed in this investigation have been described in detail previously (3). Each experiment was performed with eggs from one female and sperm from one male. Since every female contains about eight million eggs, the same biological material is sufficient for paral-

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lel series, including control and up to five or six different substances.

The following preparations were used in the experiments: styrene from Montedison S.p.A. and beta-phenylethanol from Rhône-Poulenc and styrene oxide, styrene chlorohydrin and phenyldichloroethane from Cutolo Metallorganica S.p.A. The compounds were not completely pure, which is difficult to achieve. Styrene chlorohydrin was the preparation with the lowest degree of purity; it contained 98 % of the substance.

The solubility of the test substances in sea water is low, styrene being virtually insoluble. This difficulty was overcome with the use of a 10^{-1}M presolution in absolute ethanol, which was diluted in sea water to the desired experimental concentration. Corresponding ethanol controls were carried out although ethanol in the resulting concentrations does not interfere with the results.

Moreover, it must be kept in mind that the experimental concentrations quoted are nominal and that the real concentrations were probably considerably lower, which is of significance for the interpretation of the results with, e.g., styrene.

RESULTS

Three different types of experiments were carried out: (a) The substances were added at various intervals after the fertilization of the eggs. Consequently, the substances hit the embryos at different stages of differentiation. (b) The eggs were pretreated for 5 min before being inseminated. The substances were throughly removed by washing in sea water. Consequently, no substance was present from fertilization up to the pluteus stage, and the effects were to be ascribed to the action on the egg cell during the 5 min of pretreatment. (c) The spermatozoa were pretreated for 2 min before the sperm were used for insemination. This type of experiment is analogous to experiment b. Since the spermatozoa consist mainly of the nucleus, the chances for an effect on the genome are evident.

Styrene

When the fertilized eggs or embryos were transferred to styrene of a nominal concentration of 5×10^{-4}M, the subsequent

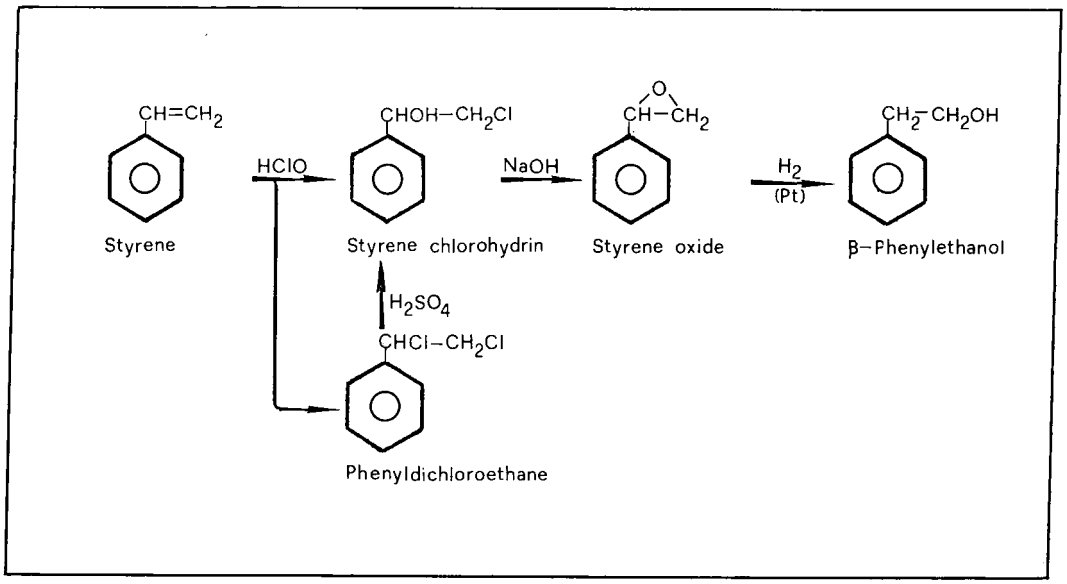

Fig. 1. The hypochlorite method for styrene oxide production.

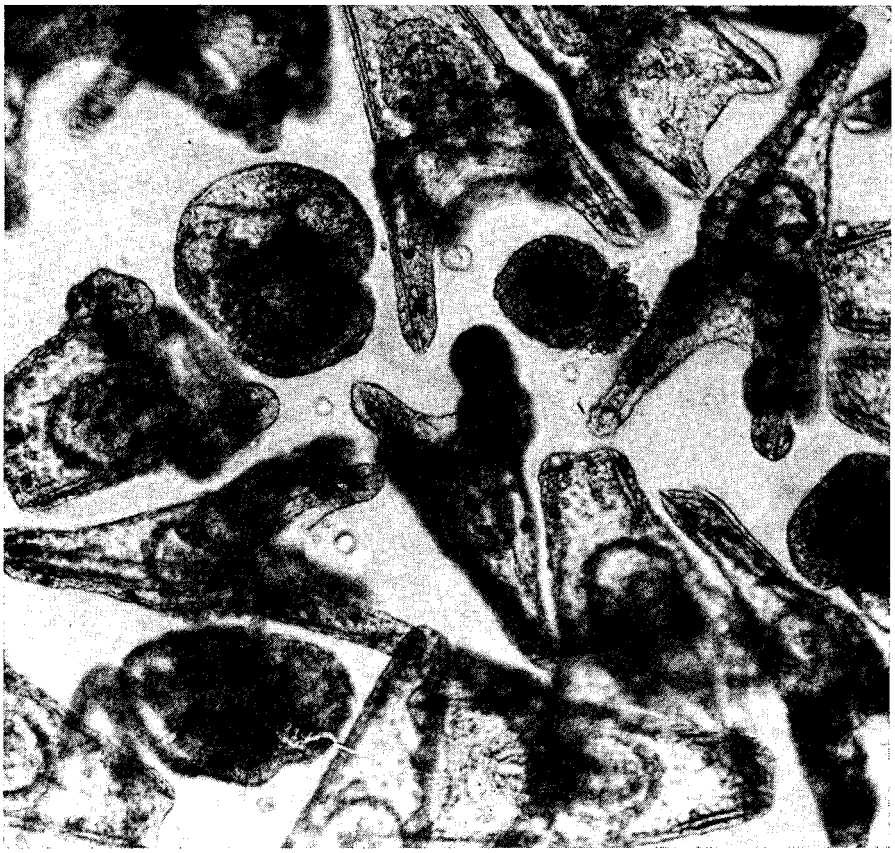

Fig. 2. Larvae generated by sperm pretreated 1 or 2 min in 10^{-4} M of styrene. The larvae are at the pluteus stage. (Magnification 250 ×)

differentiation of the embryos became entirely abnormal.

Pretreatment of the eggs in 10^{-3}M of styrene induced cytolysis of the eggs. When the nominal concentration was lowered to 10^{-4}M, cleavage of the pretreated eggs was abnormal, and, when the corresponding control had reached the pluteus stage, 100 % of the embryos of the pretreated batch were found to be pathological.

Pretreatment of the sperm in 10^{-3}M of styrene interfered somewhat with the fertilizing capacity of the sperm. However, in 10^{-4}M there were no negative effects on the fertilizing capacity of the sperm.

The embryos generated by sperm pretreated in 10^{-4} M of styrene showed abnormal cleavage, and it should again be pointed out that no substance was present during development. At the pluteus stage, $\sim$ 45 h after fertilization, 30 % of the embryos were abnormal (fig. 2).

Styrene oxide

When added after the fertilization in concentrations of 10^{-4} to 10^{-5} (nominal), styrene oxide effected disturbances of cleavage and differentiation. Cytolysis appeared within a few hours of treatment. Plutei were never formed, and differentiation stopped in a blastula or early gastrula stage.

Pretreatment of the eggs in 10^{-3}M of styrene oxide resulted in direct and total cytolysis. After pretreatment of the eggs in 10^{-4}M of styrene oxide, there was a number of damages, the differentiation of the skeleton being the most affected.

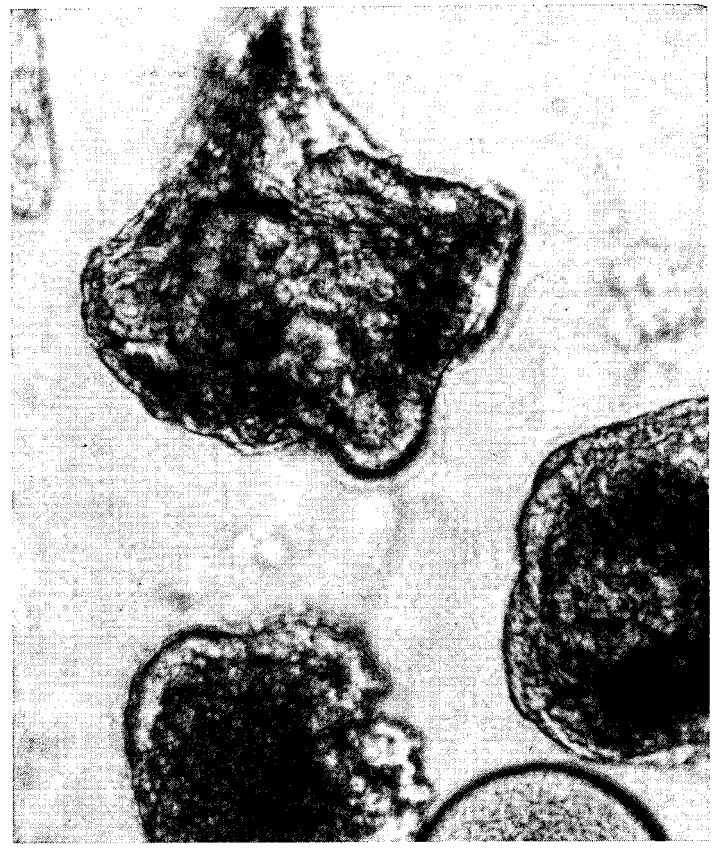

Fig. 3. Pretreatment of the sperm for 2 min in 10^{-4} M of styrene oxide. (Magnification 350 ×)

Pretreatment of the sperm in 10^{-3} to 10^{-4}M of styrene oxide (nominal concentrations) retarded fertilization and lowered the total percentage of fertilized eggs, which is normally 100. Though no substance was present, cleavage and hatching (8 to 10 h after fertilization) were affected. The differentiation of the skeleton became abnormal, showing the injuries common after pretreatment with mutagens (fig. 3).

Styrene chlorohydrin

When embryos were reared in the presence of styrene chlorohydrin (concentration range 10^{-3} to 10^{-4}M), there was a rapid blocking of differentiation. There was also an evident tendency towards cytolysis.

Pretreatment of the eggs in styrene chlorohydrin resulted in evident pathological effects. After pretreatment for 5 min in 10^{-3} to 10^{-5}M of styrene chlorohydrin the primary mesenchyme, which appears 10 to 12 h after pretreatment and fertilization, became disorganized. The resulting embryos were pathological, cell-filled blastulae.

Pretreatment of the sperm in styrene chlorohydrin affected both the rate of fertilization and the total percentage of fertilized eggs. Larvae generated by sperm pretreated in 10^{-3} to 10^{-4}M of styrene chlorohydrin showed disturbances, mainly in the differentiation of the skeleton (fig. 4). Also the rate of development was lowered when the sperm was pretreated in styrene chlorohydrin.

Phenyldichloroethane

Transfer of embryos into phenyldichloroethane had severe effects on development. In 10^{-3}M there was a rapid cytolysis. In 10^{-4}M of the substance ~ 70 % of the

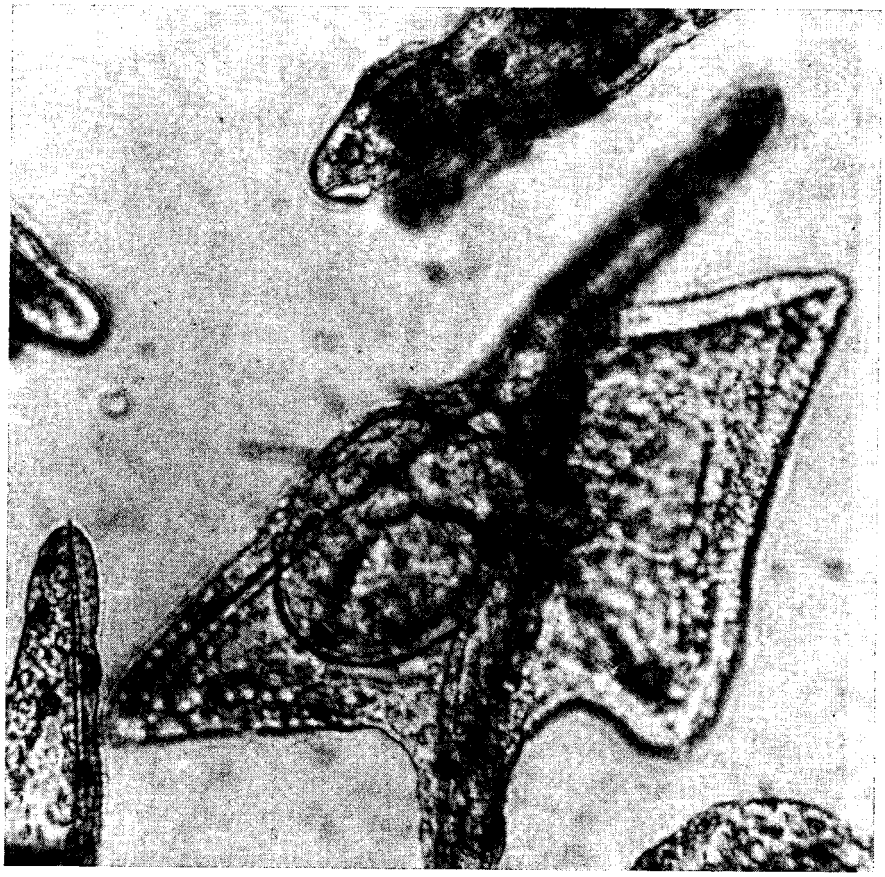

Fig. 4. Pretreatment of the sperm for 2 min in 10^{-3} M of styrene chlorohydrin. Pluteus larva with abnormal skeleton. (Magnification 600 ×)

larvae became filled blastulae. Moreover, also the rest were abnormal, although sometimes pluteus-like.

Pretreatment in 10^{-3}M of phenyldichloroethane (nominal concentration) for 5 min made the eggs unfertilizable, and the eggs soon cytolyzed. If the pretreatment was carried out at 10^{-4}M, 75 % of the eggs became polyspermic upon insemination. No normal larvae were obtained.

Pretreatment of spermatozoa in phenyldichloroethane decreased the rate of fertilization, and also the final percentage of fertilized eggs was subnormal. Of particular interest is, however, that in spite of the lowered capacity for fertilization there was a relatively high, i.e., 20 to 40 %, incidence of polyspermic eggs. Presumably the pretreated spermatozoa cannot start the cortical reaction properly, and therefore the egg surface is not sealed off to supernumerary spermatozoa.

Beta-phenylethanol

Beta-phenylethanol is relatively more water soluble than the other substances tested, and the nominal concentrations therefore probably corresponded to real experimental concentrations.

Pretreatment of the eggs or spermatozoa in this substance resulted in clear negative effects only when the concentration was kept above 10^{-3}M. Below this concentration level the effects were not appreciable.

When beta-phenylethanol was added after fertilization and was present during development, clear negative effects on differentiation were observed. The blasto-

coel became filled, which prevented further development. If added after gastrulation, beta-phenylethanol caused disturbances of the differentiation of the skeleton.

DISCUSSION

When the results of this study are summarized, it appears that the direct toxicity of the tested substances is relatively low. Although the real concentrations in the experiments are probably considerably lower than the nominal level, styrene and styrene oxide and the chlorinated derivatives caused severe effects during the time of treatment.

Decisive results, and probably also the most informative ones, were obtained in the experiments in which the eggs or the spermatozoa were pretreated before being used in fertilization. In these experiments no substance was present from fertilization up to the end of the experiments when the embryos had developed into self-maintaining pluteus larvae. Consequently, the effects registered were brought about through the few minutes of contact between the substance in question and egg or sperm.

It is also important to note that the effects elicited by pretreatment are not to be described as general pathology. On the contrary, it is evident that the disturbances appear long after fertilization, in most cases 12 to 25 h afterwards, and that the damages inflicted strike restricted and specific strata in the germinal layers, as for instance the skeleton.

We have tested a number of known mutagens/carcinogens in this test system, and we have observed that the "weak" mutagens which do not inflict severe chromosome aberrations or chromatid breaks give results similar to those found in this investigation. We have therefore arrived at the conclusion that this test model, and particularly the sperm pretreatment experiment, is suited for the detection of directly acting mutagens.

Of particular interest are the results obtained with styrene, which clearly indicate that this substance interferes with the genome.

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