The teratogenic action of thalidomide on marine fish larvae

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Summary. The effect of thalidomide on the differentiation of 4 species of marine fishes was investigated. Thalidomide interferes with the development of the skeleton. It is indicated that thalidomide acts as a mutagene.

The tragic consequences of the use of thalidomide have induced a number of investigations of the action of this drug[1–5]. In spite of this, the reasons for the teratogenic effect are still obscure. In an earlier study, it was indicated that thalidomide changes the genome and particularly the part of the genome connected with the differentiation of the skeleton. We also found that the negative effects of thalidomide on the differentiation of the mesoderm were completely neutralized if the drug was mixed with DNA before it was applied in the experiments[6]. Since thalidomide presumably acts on the genome, we infer that it must also act on a wide variety of organisms, and we therefore started the present investigation using gametes and embryos from species of marine fishes as material.

Material and methods. The investigation was carried out at Stazione Zoologica, Naples, using Crenilabrus pavo Cuv. et Val. and C. tinca (Brünn) as material. At the Marine Biological Station of Tromsö, in northern Norway, we investigated the species Gadus morhua L. and Pleuronectes platessa L. In Naples, the experiments were carried out at a temperature of 18 °C and in Tromsö at 5 °C, and therefore the rate of development is considerably different. The embryos of the Crenilabrus species hatch in about 1 week, whereas the Gadus-larvae become free-swimming within 3 weeks and those of Pleuronectes within 4 weeks after fertilization.

Thalidomide dissolves in sea water to a maximum concentration of about 10^{-4} M. Sea-water solutions are instable and retain the teratogenic activity for a few h only[6]. If a long-term exposure to the drug is needed, thalidomide must be renewed every 3rd or 4th h. In most of our experiments we pretreated the sperm or the eggs before insemination. The time of pretreatment was 2 or 5 min respectively. In these experiments thalidomide was not present in the sea water medium after insemination and consequently did not interfere directly with cleavage and differentiation.

Results. A. Pretreatment of the gametes. It was found difficult to influence the differentiation of the egg by pretreatment of the egg in a 10^{-4} M solution of thalidomide before fertilization. This may be because the chorion of the unfertilized fish egg is tough and impermeable, as has been indicated in experiments with a number of other

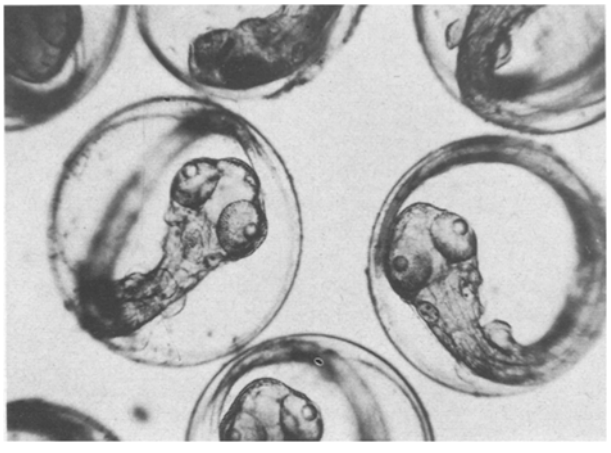

Fig 1. Gadus morhua. Control larvae just before hatching. × 200.

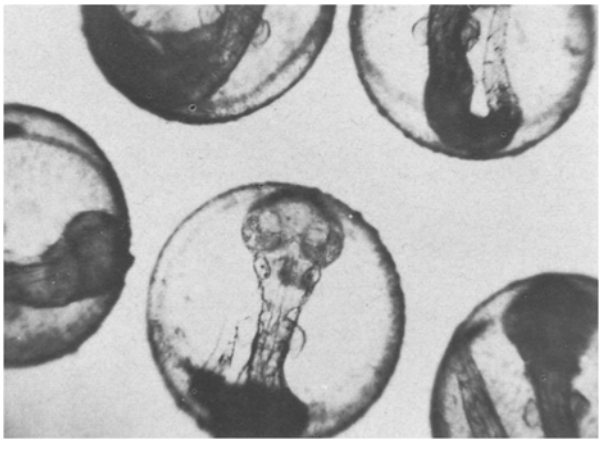

Fig. 2. Larvae from the same parents as in figure 1. Pretreatment of the sperm for 2 min in 10^{-4} M thalidomide. The tail is bent and defective and the development of the eye-lenses is impeded. × 200.

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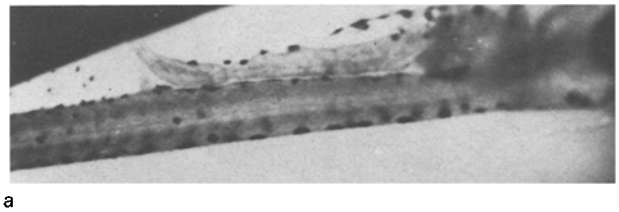

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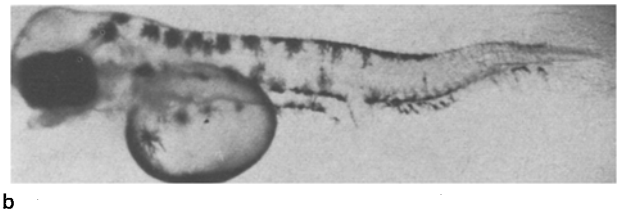

b

Fig. 3. Crenilabrus tinca. a) Control larva. b) After treatment for 3 h in 10^{-5} M thalidomide from 6 h after fertilization. × 200.

substances. Another cause of this non-susceptibility may reside in the egg nucleus, since the fish egg completes the maturation divisions after the spermatozoon has entered the egg. However, in 2 experiments with Crenilabrus pavo, we observed that about one-fourth of the pretreated eggs gave rise to embryos with disturbances of the skeleton. When, on the other hand, the spermatozoa are pretreated (2 min in 10^{-4}–10^{-5} M thalidomide) the results were very clear, and in agreement with those previously obtained with sea urchin larvae[6]. After pretreatment of the sperm, the differentiation of the notochord becomes disturbed. Also the general development of the larva is clearly affected and the development of the various larval organs is delayed or suppressed. A very evident effect of pretreatment of the spermatozoon is the defective differentiation of the distal part of the notochord (cf. figures 1, control, and 2, thalidomide).

B. Treatment with thalidomide after fertilization. A treatment for only 3 h, started at various intervals after fertilization, brought about severe disturbances of the development of the notochord and the resorption of the yolk sac was likewise affected. There was, moreover, an accumulation of pigment; figures 3a (control) and 3b (10^{-5} M thalidomide for 3 h) show the effect of thalidomide on the differentiation of the embryo. Cleavage was influenced negatively and hatching became delayed, which indicates that not only the mesoderm is affected by the drug. Though the treated embryos hatch into free-swimming larvae, the lethality is high and no adult animals are likely to be formed.

Discussion. It is a remarkable fact that the response to treatment with thalidomide during embryonal life of warm-blooded animals displays a rich variation, which is evidently correlated with the particular strain of animals used in the experiments[2–4]. We introduced gametes and embryos from sea urchins as a material for testing toxicity on the cellular and embryonal level[6,7].

The present experiments with marine fishes corroborate the results from our previous work on echinoderms. The species of fishes used are very different and they live and reproduce under very different environmental conditions. The response to thalidomide was uniform, and not species-related, and the main morphogenetic effects were concentrated to the differentiation of the mesoderm. The full effect was obtained already by pretreating the spermatozoon for only 2 min in a 10^{-5} M solution of thalidomide. Since the spermatozoon mainly consists of a nucleus, it is warranted to conclude that thalidomide interacts with the genome, and particularly with the genes which govern the differentiation of the skeleton.

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Simple theoretical criterion of chemical carcinogenicity

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Summary. The correlation between the average quasi-valence number and carcinogenicity of organic compounds has been established and discussed.

In our recent work[1], we have established the correlation between the pseudoatomic potential[2] and carcinogenicity. The complex form of the pseudoatomic potential, employed in the previous work, contained Coulomb interaction and Pauli repulsion components, and it was rather difficult for calculation by non-specialists.

Since in this potential the average quasi-valence number Z*, defined as:

$$Z^* = \frac{\sum_{i=1}^{m} N_i Z_i}{\sum_{i=1}^{m} N_i} \qquad (1)$$

where N_i is the number of atoms of the i-th type in the given molecule, Z_i is the number of valence electrons in the atom of the i-th type[3], and m is the number of chemical elements in the molecule, plays a very important role[4,5], we decided to look for the possible existence of a correlation between the average quasi-valence number and carcinogenicity of chemical substances.

Calculated values of the average quasi-valence number for about 400 organic compounds of known carcinogenic activity strongly correlate with their carcinogenic properties. In the table, we present data for the analyzed compounds biologically tested in mammals. On the basis of available biological results, we have concluded that potential carcinogens have Z* values below 3.20, while noncarcinogens are characterized by Z* above 3.20. The borderline value of 3.20 has been chosen on the basis of empirical biological data. The Lyon (International Agency for Research on Cancer) criterion for carcinogenicity has been used in this work.

The classification of about 400 organic compounds[6] into 2 categories: potential carcinogens and noncarcinogens is connected with an error of about 8%. Corresponding error for compounds tested in mammals and reported in Lyon's monographs is only 5%.

In connection with the above average quasi-valence number criterion, one should notice the following:

a) The criterion is based on the use of molecular formula and it is insensitive to the effects of isomerisms.

b) Investigations of the primary molecular form alterations due to metabolic processus have shown that the average quasi-valence number of original molecule is not changed by more than 10%. This explains some of the

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6 In the table we included all those substances for which we found well established biological data obtained from mammalian systems.