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EFFECTS OF ACTINOMYCIN *D* ON MORPHOGENESIS

by

J. BRACHET H. DENIS,
(Université Libre de Bruxelles)

1963

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It was found that actinomycin *D* very strongly inhibits the differentiation of the nervous system, especially the brain : strongly microcephalic embryos can easily be obtained in this way. Cytological studies indicate that actinomycin *D* obliterates the cephalo-caudal gradient of RNA synthesis in the gastrula and neurula stages.

In *Acetabularia*, actinomycin *D* completely inhibits the regeneration of nucleate halves, but not that of anucleate halves.

These results strongly suggest that the production of a messenger — like RNA by the nucleus really — is a very important factor in morphogenesis in plants as well as in animals.

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Effects of Actinomycin *D* on Morphogenesis

THE possible role in morphogenesis of ribonucleic acids (RNA) produced by the cell nuclei (presumably 'messenger' RNA's) has been discussed recently by one of us[1]. In short, it was suggested that, in a relatively simple system such as the regenerating unicellular alga *Acetabularia*, the 'morphogenetic substances' of Hämmerling[2] could be equated to messenger (informational) RNA; however, these substances might as well be proteins, the synthesis of which is controlled by a specific RNA of nuclear origin. The situation is obviously much more complex in the case of embryonic differentiation; we have suggested[1] that, in amphibian eggs, synthesis of messenger RNA does not begin before gastrulation. At that stage of development, the pre-existing polarity gradient in the distribution of ribosomal RNA would become activated by messenger RNA's produced by the nuclei: as a result, RNA and protein synthesis would proceed further along the well-known cephalocaudal and dorsoventral 'morphogenetic gradients.

Since actinomycin *D* strongly and selectively inhibits the production of nuclear RNA by combining with the DNA template[3,4], it was of interest to test the possible effects of this antibiotic on morphogenesis in both *Acetabularia* and developing eggs. This communication summarizes the results so far obtained.

(1) *Acetabularia mediterranea*. Since it is known[5,6] that the production of the 'morphogenetic substances' still proceeds in the dark during 1–2 weeks and since actinomycin solutions are fairly sensitive to light, the following experimental conditions were adopted: nucleate and anucleate fragments of large algae almost ready for cap formation, as well as young whole algae (10–15 mm long), were placed in actinomycin (20–1 μg/ml.) containing sea-water for 8–10 days in the dark. By that time, growth and whorls formation had stopped in the controls (darkness) as well as in the actinomycin-treated algae. The algae, which looked exactly alike, were then placed in the light (actinomycin being renewed every 5 days) and cultivated during 2–3 weeks.

Three independent experiments have shown that actinomycin inhibits growth and morphogenesis much more strongly in the nucleate algae than in the others. This effect is more easily obtained at the highest concentration used (20–10 μg/ml.) than at the lowest ones. In anucleate halves, cap formation is usually slightly delayed; but in the experiments made at the 10 μg/ml. level, all anucleate fragments formed caps which grew at a slower pace than

Fig. 1. *Pleurodeles* eggs cultivated for 4 days. Left, actinomycin *D* 10 μg/ml. ; right, control

in the controls. The results of these experiments are practically identical with those obtained in this laboratory by de Vitry[7] when she treated *Acetabularia* halves with fluorodeoxyuridine. This analogue has now been found to inhibit strongly the transfer of nuclear RNA to the cytoplasm of the algae (de Vitry, unpublished). While the results of our experiments stand in agreement with the hypothesis stated here, the reasons for the unexpected inhibition of growth in the caps formed by the anucleate fragments in the presence of actinomycin remain to be studied.

(2) *Embryonic development.* Most of the experiments have been performed on amphibian eggs (*Pleurodeles* and *Xenopus*), using concentrations of actinomycin ranging between 10 and 1 μg/ml. (in the dark). In both species, cleavage is completely unaffected ; that this negative result is not due to lack of penetration of actinomycin was shown by experiments in which the antibiotic was microinjected in fertilized eggs : again, cleavage proceeded normally. Cleavage of fertilized sea urchin eggs also remained unimpaired by the addition of actinomycin. In *Xenopus*, treatment of the eggs with actinomycin at the highest concentrations (10 μg/ml.) used always produced exogastrulation, while gastrulation was usually normal in *Pleurodeles*. If, during gastrulation, the vitelline membrane of *Pleurodeles* embryos is removed, very remarkable results can be obtained in the presence of actinomycin : at 10 μg/ml., neural induction quickly stops, although the embryos survive for several days and elongate somewhat. The nervous system is practically absent, even in sections, in 80 per cent of the cases (Fig. 1). The head can scarcely be recognized from the tail and its basophilia is abnormally

low. Chorda and somites are generally present, but they are very poorly differentiated. At lower concentrations (5–2·5 μg/ml.), the nervous system is present; but microcephaly, microphthalmy and even anophthalmy are the rule. The cephalocaudal RNA gradient is no longer recognizable cytochemically; it can even be reversed. The development and basophilia of the nucleoli are highly variable. Many nuclei undergo pycnotic degeneration.

Preliminary experiments on explanted chick embryos have given results which are in substantial agreement with those obtained with the amphibians: again, actinomycin strongly inhibits the differentiation of the nervous system, especially that of the brain.

These results stand in excellent agreement with the hypotheses which have been presented earlier in this communication: they suggest that the production of nuclear (presumably messenger) RNA must be of little importance until cleavage ends; inhibition of its synthesis with actinomycin results in the abolishment or considerable reduction of cephalocaudal and dorsoventral differentiation. But extensive biochemical studies are obviously required before the RNA fraction, the synthesis of which is sensitive to actinomycin, can be definitely identified as messenger RNA.

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