ACTINOMYCIN D INHIBITION OF CELL DIFFERENTIATION
IN THE AMPHIBIAN SUCKER

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BRACHET and DE_ (1963) and DENIS (1963) recently reported that the polypeptide antibiotic actinomycin D interferes with differentiation in amphibian embryos. Eggs of Pleurodeles and Xenopus (species not stated) treated with the inhibitor in a concentration of 10 µg/ml cleaved but the resulting embryos were stunted, showed poor differentiation of notochord and somites, and possessed practically no nervous system. PATRICIA BAKER obtained similar results (unpublished) in this laboratory using the treefrog, Hyla regilla. Flickinger (1963) observed that early gastrulae of Rana pipiens exposed to actinomycin D (7.5 µg/ml) for two days and thereafter maintained in Niu-Twitty solution developed into non-motile larvae in which the brain and spinal cord were often absent and axial muscle was poorly differentiated. Other structures, however, such as notochord, sense organs, heart, etc. were normal. The above authors attributed the inhibition of differentiation to a primary blockage in DNA-dependent RNA synthesis.

To my knowledge no observations have been published as yet on the effect of actinomycin D on the fine structure of differentiating amphibian cells, other than a preliminary report (Eakin 1963b) of the present study of the inhibited oral sucker or adhesive disk of the anuran larva.

Materials and Methods

Embryos of the Pacific Treefrog, Hyla regilla, used in this investigation were collected from ponds and streams near Berkeley. Beginning neurulae were removed from the investing jelly and fertilization membranes, washed in several changes of sterile Niu-Twitty solution (Niu and Twitty 1953), and bisected into anterior and posterior halves to facilitate penetration of the inhibitor. The anterior pieces were then cultured in total darkness at 20°C for 48 hours in actinomycin D (Courtesy of Merck, Sharp and Dohme) ranging in concentration from 2 to 20 µg/ml of Niu-Twitty solution. The medium was rendered sterile by filtration. At the conclusion of the period of treatment the embryos were washed, examined, and dissected. The developing suckers or sucker anlagen, depending upon the degree of differentiation, were excised and fixed in Dalton’s solution (Dalton 1955) at pH 7.2 for 1—2 hours at 0°C. The samples were then quickly dehydrated in 50, 70, and 100 per cent ethanol, embedded in the epoxy resin, Epon (Luft 1961), sectioned with a Porter-Blum microtome with diamond knife, mounted with a Westfall-Healy section mounter (Westfall and Healy 1962), and stained with lead citrate (Reynolds 1963). Electron micrographs were made with an RCA-EMU-3 G microscope.

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Results

1. General Morphology

Embryos subjected to dosages of actinomycin D of 8 μg/ml or higher were usually arrested at the end of neurulation and became highly necrotic or completely disintegrated. Those in 4—6 μg/ml developed to an early tailbud stage (stage 17, EAKIN 1947) but exhibited necrosis of neural tube and mesoderm. Oral suckers were most frequently single median elevations that were often no more pigmented than the surrounding epidermis. Specimens in 2 μg/ml cultures were more healthy and better developed, some attaining a middle tailbud stage (stage 18) at the end of 48 hours. Their suckers ranged from one pigmented thickening of the ectoderm through a dumbbell-shaped rudiment to paired disks, some of which approached the controls in normalcy of development. The latter reached the late tailbud stage (stage 19), with well differentiated and functional adhesive disks, after two days of development under conditions identical to those imposed on the experimental embryos except that actinomycin was not added to the culture medium.

Cells of the strongly inhibited sucker retained the cuboidal shape of simple ectodermal elements instead of becoming columnar like those in the adhesive disk of the control. Nuclei of the former were often situated close to the outer cell surface; normally they lie near the basal ends of the cylindrical secretory units. Fewer pigment granules were present in the inhibited cells than in those of the normal sucker and they were predominantly superficial in position. In the disk of the control, on the other hand, pigment granules were largely sub-surface (see Figs. 5 and 8 of EAKIN 1963a), the layer of cytoplasm beneath the plasma membrane being packed with secretion. Secretory activity was usually absent in the strongly inhibited sucker and consequently the little hillocks were non-adhesive. Normally the sucker of *Hyla regilla* becomes sticky in the late tailbud stage (EAKIN 1963a).

2. Fine Structure

*Endoplasmic reticulum.* In the control (late tailbud stage) the endoplasmic reticulum consisted of long cisternae studded externally with ribosomes (er, Figs. 1—3; see also EAKIN 1963a). In the poorly differentiated sucker of an embryo treated with actinomycin, however, this organelle was a loose aggregation of large smooth vesicles (er, Fig. 4). The stage of differentiation of the reticulum in the latter was very similar to that (er, Fig. 6) in the disk anlage of the normal early tailbud embryo (stage 17).

*Ribosomes.* Control and experimental animals were strikingly different in the number of ribosomes present in the cells of the developing adhesive disk. As just noted the endoplasmic reticulum of the former was coated with a layer of granules assumed to be ribonucleoprotein (RNP). The primitive reticulum of the latter, on the other hand, was largely devoid of ribosomes. Only an occasional vesicle showed short chains of granules attached to segments of its outer surface. The same picture was observed in the control at 24 hours after the beginning of the experiment by which time normal development had proceeded to the early tailbud stage. Unfortunately Figs. 4 and 6 do not illustrate such vesicles.