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Effect of Localized Increased Temperatures on a Frog Egg (*Hyla regilla*)

SHELDON MARGEN AND A. M. SCHECHTMAN.

From the Department of Zoology, University of California at Los Angeles.

It is well known that the amphibian organizer center (dorsal blastoporal lip) induces a neural plate in any presumptive ectoderm with which it may come in contact. It has been claimed that neural plates may also be induced in presumptive ectoderm by application of a thermal gradient (Gilchrist,[1] Castelnuovo[2]) as would be expected on the basis of Child's[3] gradient theory. Our experiments on the tree frog *(Hyla regilla)* have not thus far substantiated the latter work.

The apparatus used, we believe, is easily the most accurate yet applied to this study of the effects of thermal gradients on amphibian eggs. The thermodes used were made of 4 coils of platinum wire 1/500 of an inch in diameter, insulated with a thin coat of lacquer, and heated with current from a storage battery. The temperature could be regulated by a resistance box. The coils of platinum wire were carefully placed against the upper surface of the egg by means of a micro-manipulator set. It was possible to observe the eggs constantly during the period of heating. The eggs were also vitally stained where the thermode was placed, in order to be certain that no movement took place.

The temperature applied to the egg surface was ascertained directly by the use of a micro-thermocouple and galvanometer.

The thermal death-point of the cells was found to lie at about 36-38°C. Our experiments to date have been concerned with the application of temperatures close to the thermal death-point (27-36°C). On the basis of the physiological gradient theory of Child, these high temperatures ought to be most effective in inducing secondary neural structures.

Examination of the external surfaces of the heated eggs has thus far revealed no case of clear neural induction. The heated portion is usually more advanced in development than the rest of the egg. Thus, in one case in which the ectoderm of the face region was heated, the typical prominences of the suckers were present anteriorly

1 Gilchrist, F. G., *Physiol. Zool.*, 1928, **1**, 231.

2 Caltelnuovo, 1932, quoted in Dalcq, A., and Pasteels, J., *Bull. de l'Acad. royale de Médecine de Belgique*, 1938, VIe série, tome 3, 261.

3 Child, C. M., *Protoplasma*, 1928, **5**, 447.

while the neural folds were not yet closed in the middle and posterior parts of the body. One entire neural fold may arise on the heated side before any fold appears on the opposite side or in the control (non-heated) eggs.

The specimens we have thus far examined in sections show no clear neural induction. However, there are ectodermal thickenings and in some cases the heated ectodermal cells are columnar in form, whereas non-heated ectoderm of the same egg is composed of low cuboidal or flattened cells. Whether this may represent a slight attempt at neural induction, is doubtful at present. We wish to emphasize that the implantation of organizer material in *Hyla regilla* results in perfectly clear induction in almost 100% of the cases (Schechtman[4]).

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Experiments on Anus Formation in a Frog Egg.

A. M. SCHECHTMAN.

From the Department of Zoology, University of California at Los Angeles.

The anus arises in *Hyla regilla*, as in most vertebrates, as an ectodermal (proctodeal) pit which subsequently becomes perforated to form the anus. We have attempted to analyze the factors responsible for this differentiation.

The presumptive proctodeal ectoderm of the early gastrula has no capacity to form a proctodeal pit. This ectoderm was transplanted to the chest region, where it formed smooth epidermis. The anterior ectoderm (of the face and chest) were transplanted to a posterior position, just over the ventral blastoporal lip, and here developed into very clear proctodea. Evidently the ventral lip contains some factor or factors responsible for the appearance of the proctodeum, as was previously concluded.[1] When the ventral lip is implanted into the blastocoele of another early gastrula, the host ectoderm develops a proctodeum usually on the side or belly.

In 2 series of extirpation experiments the mesoderm or the entoderm of the ventral lip was removed from the early gastrula. In the absence of the entoderm a proctodeum forms in all cases. After re-

4 Schechtman, A. M., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 236.

1 Schechtman, A. M., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 236.