

Competence for Neural Plate Formation in *Hyla* and the So-called Nervous Layer of the Ectoderm.*

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The appearance of the neural plate in Amphibia is known to be dependent upon two main factors: First, there must be an inducing stimulus which normally emanates from the chorda-somite mesoderm; and secondly, the reacting ectodermal cells must be in a susceptible condition. This latter condition is now commonly referred to as "competence" for neural plate formation,¹ and has vanished by the time the egg reaches the early neurula stage.²

In an Anuran egg, *Hyla regilla* the competence of anterior and posterior portions of the presumptive epidermis was compared at several successive stages of development. The operative method was somewhat different from the "Einsteckungsmethode" of Mangold, in which the dorsal blastoporal lip is simply inserted into the blastocoele. In the present experiments the presumptive epidermal explant was first allowed to fuse firmly with the dorsal lip by placing the 2 pieces in contact on the floor of the operating dish. The combined explants (presumptive epidermis plus dorsal lip) were then implanted in a uniform position in another gastrula (Fig. 1). This method assures us that all the pieces of ectoderm remain in contact with the dorsal lip for an equal length of time and that they develop

TABLE I.
Summary Table of the Percentage of Neural Plates Induced in Anterior and Posterior Presumptive Epidermis by Dorsal Blastoporal Lip of the Early Gastrula.

Stage	Region of Presumptive Epidermis	No. of Eggs	No. of Neural Plates Induced	% of Inductions
Early Gastrula	Anterior	50	49	98
	Posterior	46	44	95
Mid-Gastrula	Anterior	24	19	79
	Posterior	20	7	35
Late Gastrula	Anterior	21	2	9.5
	Posterior	19	0	0
Early Neurula	Anterior	13	0	0
	Posterior	16	0	0

* Aided by a research grant from the University of California.

¹ Waddington, C. H., *Philos. Trans. B*, 1932, **221**, 211.

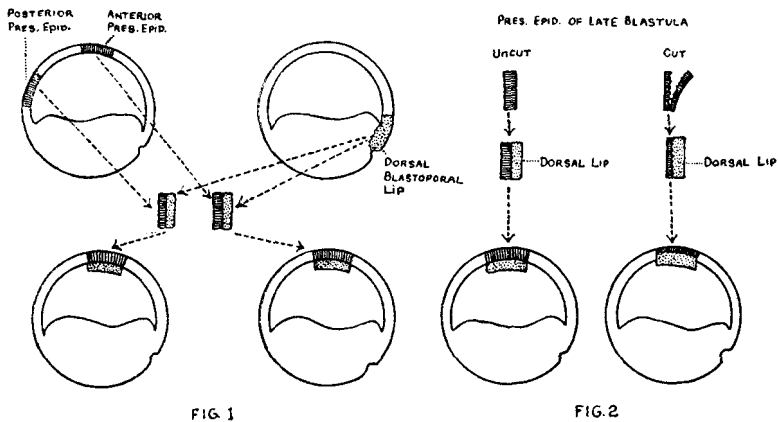
² Mangold, O., *Arch. f. Entw-mech.*, 1929, **117**, 586.

in the same position in the host. Close to 100% neural plate inductions are obtained *consistently* when the presumptive epidermis is from an early gastrula.

Table I shows the results obtained at several stages of development when anterior and posterior epidermis are tested.

This table includes only perfectly clear cases of neural induction in which the ectodermal cells and their nuclei were distinctly elongated and closely packed to form a neural plate or tube.

In his work on urodeles Machemer³ did not report any definite data for the competence of anterior and posterior presumptive epidermis in the *early* gastrula stage. He makes the statement, however, that he did a few experiments on this stage and these indicated that the *posterior* presumptive epidermis was apparently more competent than the anterior (p. 450). Our results with *Hyla* (Table I) show that there is little or no difference in the capacity of anterior and posterior presumptive epidermis of the *early* gastrula. However, in the *middle*-gastrula stage, when the yolk plug measures about half the diameter of the egg, the posterior ectoderm is distinctly less competent than the anterior. This confirms Machemer's work on this stage in the Urodeles.³ Table I further shows that when the egg has reached the advanced gastrula stage the posterior presumptive epidermis has completely lost its competence, but the anterior presumptive epidermis occasionally reacts. Presumptive epidermis from the neurula always fails to react.



Among the recent hypotheses as to the factors involved in loss of neural plate competence, is the ingenious one of Dettlaff⁴ to the

³ Machemer, H., *Arch. f. Entw-mech.*, 1932, **126**, 391.

⁴ Dettlaff, T., *Zool. Jahrbücher*, Abt. allg. Zool. u. Physiol., 1936, **57**, 203.

effect that the inner layer of presumptive epidermis cells (the so-called nervous layer) gradually migrates away from the region of the presumptive epidermis toward the future neural plate. According to Dettlaff competence vanishes because it depends upon the presence of this inner layer of cells which migrates away during gastrulation. We have still another possible explanation of the loss of competence in the thinning of the ectoderm which goes hand in hand with its loss of competence. Waddington⁵ had advanced evidence that mere thinning of the ectoderm plays a dominant rôle in the competence for lens; this suggests the possibility that thinning may also affect competence for neural plate formation.

The above two hypotheses were tested by the following simple experiment. In the advanced blastula, when the roof is still quite thick, a square of presumptive epidermis was removed and then cut in half *parallel* to the surface of the egg. The outer pigmented half of this explant was then combined with dorsal lip from the early gastrula and implanted into another egg (Fig. 2). Thus the inner layers of cells (the "nervous layer") stressed in Dettlaff's hypothesis were to a large extent eliminated. At the same time the piece of presumptive epidermis was reduced to about half its normal thickness. In the controls the ectoderm remained of normal thickness ("uncut," Fig. 2).

The results are perfectly clear (Table II): The thin layer of presumptive epidermis is as competent as the normal layer, as shown by the percentage of neural inductions. Furthermore, the induced neural plates develop simultaneously in both groups. Machemer³ showed that less competent presumptive epidermis takes longer to differentiate into neural plate than the more competent.

TABLE II.
Percentage of Neural Plates Induced in Presumptive Epidermal Layers of Normal and Half-normal Thickness.

Thickness of the Ectoderm	No. of Eggs	No. Induced Neural Plates	% of Inductions
Normal	16	14	87.5
Half-normal	18	16	89

It cannot, of course, be supposed that in this experiment all the cells which constitute the so-called nervous layer of the ectoderm were removed. *It is certain, however, that a great number of these cells were removed and that the competence of the presumptive epidermis was not thereby affected.* We must conclude, therefore, that

⁵ Waddington, C. H., *J. Exp. Biol.*, 1936, **13**, 86.

neither loss of the nervous layer nor mere thinning of the presumptive epidermis are responsible for its loss of competence.

Summary. 1. The anterior and posterior presumptive epidermis of the early gastrula of *Hyla regilla* possess essentially the same degree of competence for neural plate formation. 2. In the middle and late gastrula stages the posterior presumptive epidermis is less competent than the anterior. 3. Thinning of the ectoderm by removal of at least a large part of its inner layers of cells (the so-called nervous layer) in the advanced blastula stage does not impair the competence of the ectoderm.

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Influence of Vitamin D in Experimental Lead Poisoning.

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In the present investigation a study was made of the effect of the antirachitic vitamin upon the concentration of lead in the blood and upon the amount of lead deposition in the bones. This was undertaken because it has been noted previously that the severity of both experimental and clinical lead poisoning is increased under the influence of vitamin D,^{1, 2, 3} or during the summer months when the sunlight is rich in ultraviolet rays.

A series of young rats, 23-26 days of age, was placed on a lead-containing diet. Half of these animals received approximately 33 Steenbock units of viosterol in halibut liver oil daily (Mead Johnson) after the first 5 days on the lead-containing diet. The composition of the diet is given in Table I. It is essentially a rickets-producing diet where 3% of $\text{Pb}(\text{OH})_2 \cdot 2\text{PbCO}_3$ was substituted for 3% of CaCO_3 . At the end of the experimental period the concentration of lead in the whole blood and the amount deposited in the bones was determined. The results of the experiments are presented in Tables II and III.

¹ Shelling, D. H., *Proc. Soc. Exp. Biol. and Med.*, 1932, **30**, 248; Shelling, D. H., and Hopper, K. B., *Bull. Johns Hopkins Hospital*, 1936, **58**, 193.

² Krehbiel, O. F., personal communication to Dr. Shelling from Dr. C. E. Willoughby of the Chemical Experiment Station, E. I. DuPont de Nemours Company.

³ Blackman, S. S., *Bull. Johns Hopkins Hosp.*, 1937, **61**, 1.