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]).

10565 P

Experiments on Anus Formation in a Frog Egg.

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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.

moval of the mesoderm most of the eggs fail to form a proctodeum, but a few develop a complete or rudimentary proctodeal pit. This we attribute to incomplete removal of the mesoderm.

Ventral lip mesoderm from the early neurula, in which the 3 germ layers are more clearly distinguishable, was implanted into the blastocoeles of early gastrulae. The mesoderm, taken from the region just under the slit-shaped blastopore, induces a clear proctodeum in the host ectoderm. The entoderm of the hind-gut region was implanted into other early gastrulae and was not capable of inducing a proctodeum. But the epidermis of the host lying just over the implant becomes perforated, exposing the implanted piece of hind-gut.

We attempted to delimit the proctodeum-inducing region by explanting various segments of the blastoporal lips of the early gastrula. The ventral and ventro-lateral lips develop proctodea in almost all cases. The lateral lips do so in some cases (exact percentage not yet available). Dorsal and dorso-lateral lips never form a proctodeum. The inductor thus must extend approximately over the entire ventral half of the mesodermal ring. However, when only the ventral lip is removed from an early gastrula, the 2 ventro-lateral and lateral lips remaining, the embryo fails to form a proctodeum. Evidently some sort of regulatory mechanism is at work. An interesting feature of these eggs is the consistent absence of the ventral fin of the tail.

Holtfreter[2] found that an anus was induced when chorda, neural tube, entoderm, and connective tissue were present in various combinations near the ectoderm. Proctodea were also present when no neural tube, entoderm, or chorda lay near the ectoderm, although apparently in all cases some connective tissue was present. Risley[3] has advanced evidence that connective tissue alone is capable of the induction; he believes it possible that the connective tissue receives its inductive capacities from the axial organs. Our work on *Hyla* makes it clear that, at least as concerns the proctodeum of the frog, there is no necessity to assume such a rôle of the axial organs. This follows from the fact that the ventral lip, which contains no chorda and is incapable of inducing a neural tube, can induce the proctodeum. Risley has also suggested that the caudal fin may be a necessary factor in anus formation. We have numerous cases in which a proctodeum was formed in the complete absence of a fin.

From the results given above we may suggest the following mechanism of anus formation: The ventral lip mesoderm induces the

[2] Holtfreter, J., *Arch. f. Entw.-mech.*, 1936, **134**, 466.

[3] Risley, P. L., *J. Exp. Zool.*, 1939, **80**, 113.

proctodeal pit in that portion of the ectoderm which happens to come into contact with it. The invagination of the pit brings it into contact with the entoderm of the hind-gut. The latter completes the process of anus formation by inducing the perforation of the proctodeal pit.

10566 P

Failure of "Vitamin K" Excess to Heal Encephalomalacia of Chicks.

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During the course of experimentation on chick rabies attention was drawn to the fact that most of the early literature did not consider the dietary factors necessary for normal nervous system development in the fowl. Pappenheimer, *et al.*,[1, 2] described the encephalomalacia present in experimental and normal flocks. This paper is concerned with the effects of a high "vitamin K"* level in an attempt to alter the encephalomalacia in chicks which is believed due to capillary alteration by some unknown factor missing from the diet.

Healthy day-old chicks were placed on deficiency diet No. 108,[1] "normal" diet No. 20[1] and controlled by a series on a ration similar to No. 20, but more satisfactory for growth. The chicks had no access to their feces. The alfalfa leaf meal extract (hexane) was homogenized in cream with the aid of a Jubilee type homogenizer and mixed with milk. Daily levels of 5 mg, 10 mg, 15 mg, and 20 mg were fed to the 4 series consisting of a total of 120 chicks on the deficiency diet.

Results. Six paralytic chicks occurred in a series of 30 on the deficiency diet without excess "vitamin K"; while 19 paralytic chicks occurred in the 4 series of the 120 chicks receiving the deficiency diet plus excess levels of "vitamin K." One paralytic chick recovered spontaneously while on the deficiency diet without excess "vitamin K" in contrast to 12 paralytic chicks receiving the excess "vitamin

1 Pappenheimer, A., and Goettsch, M., *J. Exp. Med.*, 1931, **53**, 11.

2 Goettsch, M., and Pappenheimer, A., *J. Biol. Chem.*, 1936, **114**, 693.

* The alfalfa leaf meal was generously contributed by Dr. W. Wenner, The Upjohn Company, Kalamazoo, Michigan. 1.0 cc of this extract is equal to 10 g in "vitamin K" activity of alfalfa meal.