# A NEUROSECRETORY INGREDIENT IN THE DIFFERENTIATION OF HYPOPHYSEAL BASOPHILS

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The considerable progress in the study of hypothalamic neurosecretion in the regulation of hormonal functions of the hypophysis has not diminished the importance of the problem of interaction between the neurohypophysis and the adenohypophysis. The discovery of neurosecretion has explained the morphological unification of two different elements in a single endocrine organ, the hypophysis. During early embryogenesis, the contact between the neural and the epithelial components begins at the caudal end of the hypophysis, i.e., at the region of the primary condensation of the neurosecretion; the components are subject to secretory elements which produce intermedin. The development of the other hormonal functions of the hypophysis gradually progresses in the proxial direction in relation to the strengthening of the "contact" between the basic divisions of the hypophysis. Morphologically, the longest and the most pronounced contact occurs in the region of the medial lobe of the hypophysis (in amphibians, reptiles, mammals), while the secretory apparatus of the anterior lobe of the hypophysis remains a peculiar "appendage" to the system of portal vessels which carry the neurosecretion substance from the region of the median elevation of the neurohypophysis. Morphologically, this is most pronounced in the hypophysis of birds. In birds, this vascular system does not penetrate into the median elevation, but only makes a contact with it, being located in the tuberal lobe, which in turn surrounds this portion of the neurohypophysis as a cuff.

Thus, in the caudal region of the hypophysis of many animals, the materials of the neuro- and the adenohypophysis may be in very intimate contact, even to the extent of mutual intergrowth. In the oral region, on the contrary, the two components become separated morphologically, but simultaneously become vascularly connected to each other [2]. This can be seen very clearly during the process of development of amphibians.

In the present work, we have studied the part played by the neurosecretory ingredient in the differentiation of hypophyseal basophils in the frog.

## METHODS

67 male Rana ridibunda, R. temporaria, R. arvalis, and R. Terrestris, caught during spring and summer in bodies of water in the Moscow and Voronezh regions, were used in this work. Immediately after capture, the heads of frogs were fixed in Bouin's fluid. After decalcification, the material was embedded in paraffin and 6-7 $\mu$ sagittal or frontal sections of different parts were prepared. The sections were stained with Halmi's mixture, Gab's aldehyde-fuchsin, and counterstained with Schiff's reagent in order to reveal beta-basophils. The same material from 52 frogs of different ages, in which the preoptic region was removed, was treated in the same way. The data on the biological testing of the altered hypophyses and the experimental methods have been described earlier [2,6].

## RESULTS

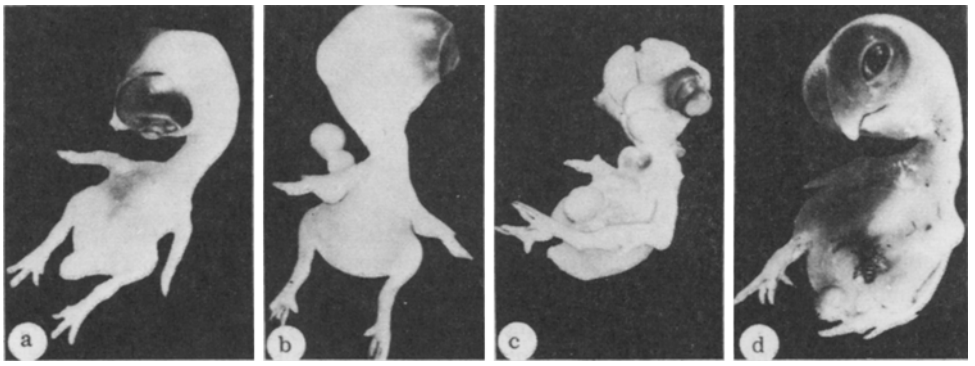
The peculiarity of structural relationships between the intermediate and the neural lobes, noted by us earlier in the hypophysis of the green frog and spadefoot toad [1], was just as clearly defined in other species of amphibia. While in R. ridibunda the neuro-intermedial "sandwich" has a fairly straight border line (Fig. 1a), in R. arvalis (as in other related species) this border line is broken as a result of mutual intergrowth of tissues of different origins

TABLE 1. Teratogenic Effect of Thalidomide on 3-Day-Old Chick Embryos

| Agent | Embryos | | |
|---|---|---|---|
| | total | dead | with defects |
| Controls | 17 | 1 (5%) | 0 |
| Dimethylformamide (1-100 mg) | 63 | 51 (81%) | 8 (12%) |
| Saline (0.1 ml) | 37 | 17 (45%) | 3 (7%) |
| Thalidomide suspension (0.001-1.0 mg) | 64 | 27 (42%) | 6 (9%) |
| Thalidomide solution (0.001-0.1 mg) | 57 | 42 (73%) | 9 (15%) |

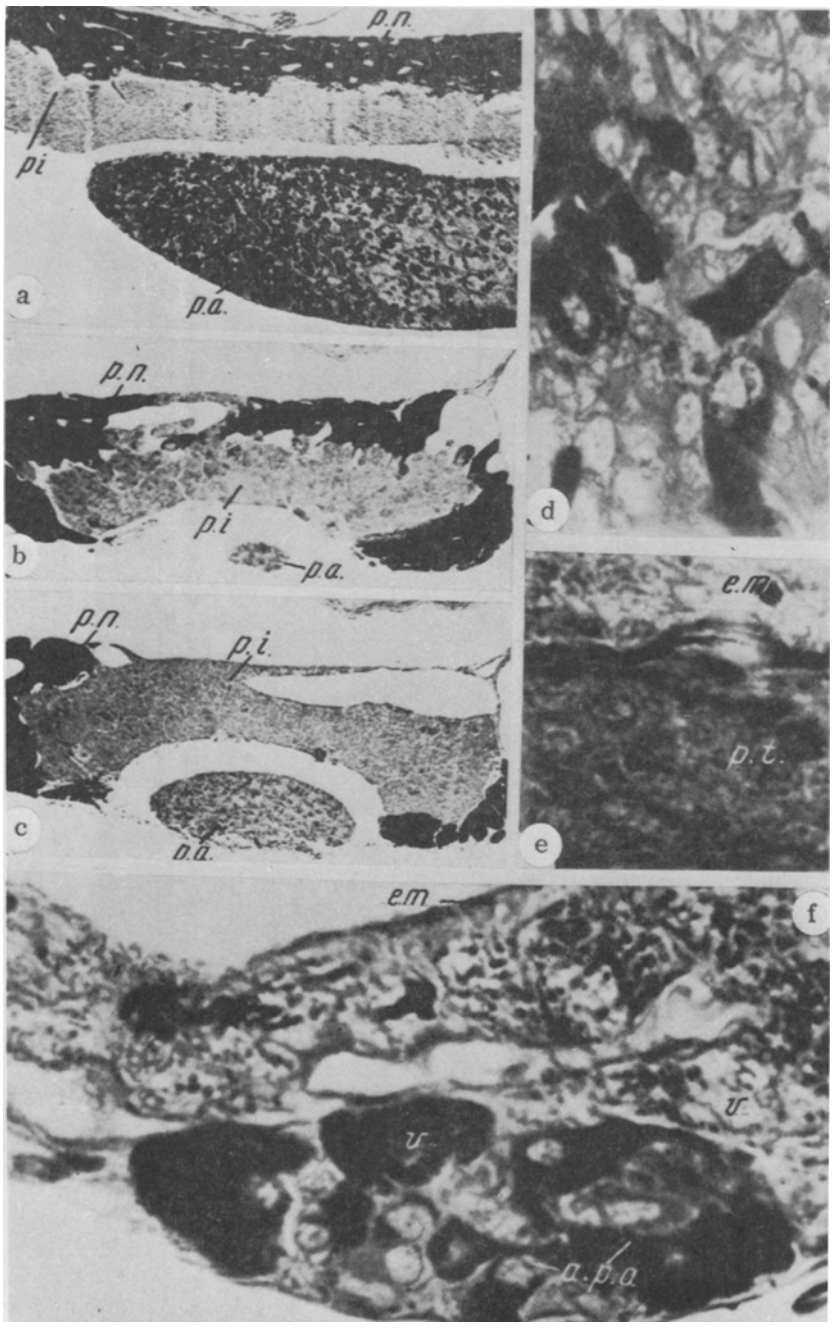
TABLE 2. Teratogenic Effect of Thalidomide on 5-14-Day-Old Chick Embryos

| Agent | Embryos | | | Defects | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | total | dead | with defects | total | brain | eyes | beak | internal organs | spine | extremities | Coefficient |
| Controls | 70 | 17 (24%) | 2 (3%) | 3 | 1 | 0 | 1 | 0 | 1 | 0 | 1,5 |
| Dimethylformamide (1-100 mg) | 59 | 47 (79%) | 7 (12%) | 14 | 1 | 1 | 2 | 4 | 3 | 3 | 2,0 |
| Normal saline (0.1 ml) | 57 | 36 (61%) | 4 (7%) | 8 | 1 | 1 | 1 | 2 | 1 | 2 | 2,0 |
| Thalidomide suspension (0.001-1.0 mg) | 98 | 82 (83%) | 20 (20%) | 70 | 4 | 9 | 14 | 11 | 17 | 15 | 3,5 |
| Thalidomide solution (0.001-0.1 mg) | 56 | 45 (80%) | 7 (12%) | 30 | 3 | 3 | 5 | 6 | 7 | 6 | 4,3 |




Teratogenic effect of thalidomide on chick embryos: a) an 8-day-old embryo (control); b) an 8-day-old embryo (defect in the development of the spine, absence of coccyx, lack of closure of anterior abdominal wall, eviscertation); c) an 8-day-old embryo (defects in the development of brain, eyes, beak, evisceration); d) a 13-day-old embryo (defects in the development of beak, spine, shortening of legs).

the development of the brain and eyes, produced a curvature of the body axis, and sometimes an inversion of position. The introduction of thalidomide in suspension led to a shortening of the body and an upset in the development of the brain and eyes. Thalidomide in solution produced the strongest degree of inhibition of development, so that 3-day-old embryos externally resembled 1-day-old ones.




Sections through the frog hypophysis: a) half a section through the hypophysis of an adult R. ridibunda; zonality in the localization of basophils in the anterior lobe; b,c) two sections through the hypophysis of R. arvalis; strong vascularization of the border regions between the posterior and the intermediate lobes; d) a portion of the anterior lobe of hypophysis of a summer R. arvalis from the zone with a large number of basophils; e) a portion of the lateral lobe (inferior); f) an abnormal island (a.p.a.) of the adenohypophysis in contact with the neurohypophysis (e.m.), located in front of the lateral lobe of R. arvalis. a, b, and c – frontal sections. Magn. 54×; d, e, and f – sagittal sections. Halmy's stain. Magn. 1200×; p.n. – posterior lobe of hypophysis; e.m. – medial elevation of the neurohypophysis; p.i. – intermediate lobe; p.a. – anterior (distal) lobe; p.t. – region of the lateral (tuberal) lobe; v. – vessels.

(figure, b and c). Moreover, a-c (figure) shows a region of the intermedial tissue from the side of the neural component, i.e., morphologically, the picture is quite opposite to that seen in lizards. The hypophysis of all species of amphibia is characterized by the fact that, in addition to a strong vascularization of the neural lobe, numerous vessels oriented in the same direction are localized at the border between the two lobes. However, here, unlike in the anterior lobe, the vessels do not grow into the parenchyma of the intermedial lobe. Often epithelial strands, which penetrate into the neural component, encircle part of the wall of the widened capillary (figure, b). As a rule, the great majority of the border vessels are found in the combined tissue envelope, half of which consists of cells of the intermediate lobe. Such an organization of the vascular network and a relatively weak basophilia of the cells of the intermediate lobe indicates that the active substance from each lobe is directed only into the vessels and renders the hypothesis of mutual "diffusion" of hormones less probable.

In the anterior lobe of the hypophysis, the picture is quite different; here the flow of neurosecretion through numerous vessels is a basic factor in the differentiation of secreting cells. We became convinced that the differentiation of basophils, rich in aldehyde-fuchsinophilic granules, is dependent on the amount of neurosecretion reaching the proximal region of the neurohypophysis. The zonality in the distribution of beta-basophils, noted by us earlier in the hypophysis of young toads [4], is especially well defined after the period of reproduction in the new species of frogs studied by us. The basophils are localized mainly in contact with the endothelium of numerous capillaries and portal vessels which enter the anterior lobe from the massive medial elevation. The irregularity in the distribution of basophils was also noted during the period preceding reproduction; this was seen in the hypophysis of the pond frog not only in sagittal, but also in frontal sections. This zonality is obliterated in individuals in which the preoptical region of the midbrain has been removed.

A study of serial sections of the hypophysis has shown that, in the majority of newly captured, sexually mature individuals, there are individual epithelial cells or small groups of such cells in the border area of the medial elevation. These cells are always basophilic and have a strongly granular cytoplasm. They are larger than the usual beta-basophils of the anterior lobe and are usually rounded, sometimes elongated in shape, and have small outgrowths. Unlike typical basophils in the anterior lobe, these cells are not subject to seasonal variations in their staining properties and of structure, related to phases of the secretion cycle [5].

Similar studies on the hypophysis of R. arvalis have revealed completely new abnormalities in 8 out of 23 individuals; these abnormalities were accessory islands of epithelial tissue distributed outside of the ventral part of the funnel or in the proximal region of the medial elevation (figure, d). Thus, in counts of 100 cells in sections of the anterior lobe, the ratio of basophils to other cells (oxyphils and chromophobes) constituted 34:66 in the zone where basophils predominate, 14:86 in the zone with the minimum number of basophils, and 58:42 in the epithelial islands (figure, f). An increase of vascularization in the region of the neural component above the abnormal formation was seen to be the rule in the localization of these islands. The basophilic cells in the epithelial strands were always large in size, some of them were pear-shaped and their cytoplasm was very strongly granular. Capillaries in such regions which proceed from the vessels in the neural component amply provide the abnormal islands with blood.

It is to be noted that the proximal regions of adenohypophysis, modified into so-called lateral lobes (which are homologous to the tuberal region of the mammalian hypophysis) are represented by plates which consist of homogeneous cuboidal, weakly staining, but not basophilic cells (figure, e). The above is typical for the tuberal lobe of mammals. The tuberal lobe, as well as the lateral plates of the hypophysis of amphibians, is a primary location of relatively large portal veins which transport neurosecretion-enriched blood from the region of the medial elevation. These vessels, when they enter into the parenchyma of the anterior lobe, form a complex network of sinusoidal capillaries.

Consequently, regions of adenohypophysis, as well as its accessory fragments, which share a complex vascular system with the neurohypophysis, become differentiated in such a manner that basophilic cells predominate in them. The number of basophils, and the expression of their basic properties, are directly related to the function of neurosecretory neurons of the preoptical nucleus. Extirpation of this nucleus leads to a considerable reduction of basophilic cells, their degranulation and to an increased water content of the cytoplasm. The latter was more strongly expressed in the younger frogs from which the source of the neurosecretion was removed.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.

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