THE HISTOPHYSIOLOGY OF THE INTERMEDIATE LOBE OF THE HYPOPHYSIS IN RELATION TO HYPOTHALAMIC NEUROSECRETION

A. A. Voitkevich and E. L. Soboleva

Department of Histology and Embryology (Head, Corresponding Member AMN SSSR Professor A. A. Voitkevich), Voronezh Medical Institute

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The differentiation of the main divisions of the adenohypophysis from the common embryonic algae, and the agreement between the structural signs and the tinctorial properties of the basophilic cells of the anterior and intermediate lobes are evidently a reflection of their functional interference. If this is true, it must obviously be more marked in the earlier stages of the phylogenetic establishment of the morphological organization of the hypophysis, i.e., in fishes and amphibians.

By means of histological examination and additional tests, N. L. Gerbil'skii [2] and B. N. Kazanskii and G. M. Persov [3] showed that in the bony fishes gonadotropic factor is formed in the intermediate lobe of the hypophysis. New findings showing that the development of the adenohypophysis and of its trophic functions is dependent on the neurohypophysis have served as the basis for wider histo-functional investigations of not only the anterior but also the intermediate lobe of the hypophysis [4, 5, 8]. Several writers [9, 10] have expressed doubts regarding the correctness of the view that the role of the intermediate lobe of the hypophysis of animals is limited to the secretion of melanophoric hormone.

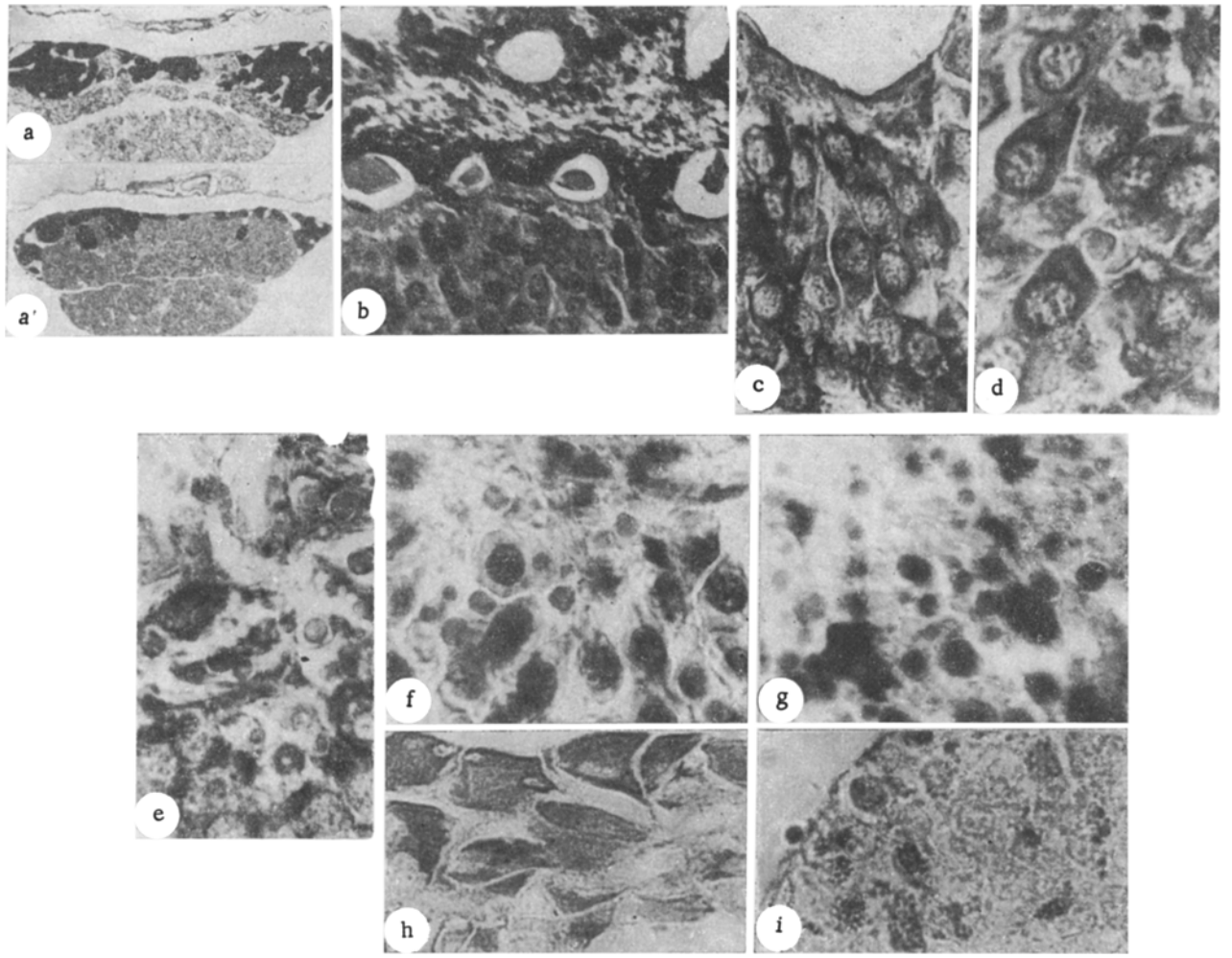
In the present article we present new data concerning the cytological changes in the intermediate lobe of the hypophysis in the period of intensive morphogenesis (multiplication and metamorphosis), unrelated to the great changes in pigmentation, but obviously due to hormonal influences. Our observations were made both on intact animals and on larvae, from which the region of the preoptic nuclei was extirpated as a preliminary measure.

EXPERIMENTAL METHODS AND RESULTS

The investigation was conducted on tadpoles and sexually mature specimens of 4 species of frog (Rana esculenta, R. ridibunda, Bombina bombina, Pelobates fuscus) and of the green toad (Bufo viridis), taken from natural ponds. Histological sections of the hypothalamus and hypophysis were stained with paraldehyde-fuchsin, chrome alum-hematoxylin with phloxine by Gomori's method, by Halmi's method, with Schiff's reagent, and by the Unna-Pappenheimer method. The intermediate zone of the hypophysis of amphibians [5-7, 9-12], in contrast to the anterior or posterior lobes, contains no blood vessels; it is in close contact with the posterior lobe, from which it receives a few nerve fibers carrying occasional granules of neurosecretion.

In the species of amphibians which we studied, the intermediate lobe of the hypophysis had no regular shape, consistency or microscopic structure. In sagittal sections from both the pond and lake frog, this lobe is shaped liked a right-angled triangle. Contact with the neural part of the stalk is limited to the apex of one of its angles. The topography of the intermediate lobe in the other two species of frog was also characterized by the wedge shape of its point of contact with the parts of the neurohypophysis in front of the posterior lobe, and by its larger area of contact with the latter. This was shown by the formation of short cell bands of the intermediate lobe, passing into the posterior lobe, best seen in frontal sections through the hypophysis in Pelobates. The intermediate lobe of the toad's hypophysis is distinguished by the fact that one side remains in contact with the neural part of the stalk over a much greater distance.

Our results showed that during metamorphosis and in the period of multiplication considerable changes take place in the microscopic picture of all parts of the hypophysis. Quantitatively, these changes were more considerable in the posterior lobe, which was greatly enlarged and strongly hyperemic. The dilated vessels were most numerous at the border with the intermediate lobe. In some species, for instance, in young specimens of Pelobates, the extent of morphological contact between the lobes was particularly increased as a result of the invasion of the posterior lobe by



Structural changes in the intermediate lobe of the hypophysis of toads and frogs. a, a') Frontal sections through the hypophysis of a young Pelobates in its 1st year: invasion of bands from the intermediate lobe into the posterior lobe, stained black on account of the presence of neurosecretion; a') this section is cut considerably more caudally than section a (stained by Halmi's method, magnification 96); b) sagittal section through the hypophysis of the pond frog before multiplication; at the smooth border between the posterior (with dark granules of deposited neurosecretion) and intermediate lobes there are hyperemic vessels (stained by Halmi's method, magnification 480); c) cells of the intermediate lobe of the pond frog in the period preceding multiplication, with their long axis pointing towards the wall of the dilated bordering vessel (stained by Halmi's method, magnification 1200); d) the same, with magnification 1800 — in individual cells the cytoplasm has become homogeneous and is stained like the delta-basophilic anterior lobe; in the center and on the right of the figure can be seen large oxyphilic droplets; e) large droplets between the cells of the intermediate lobe (the lake frog) and in the immediate proximity of the wall of a blood vessel (stained with hematoxylin by Gomori's method with phloxine, magnification 1200); f, g) the same stain, a mass of droplets in the intermediate lobe close to the bordering vessels; f) three cells with highly vacuolated cytoplasm (period of multiplication, magnification 1200 ×); h) a well marked reaction for polysaccharides in the cells of the intermediate lobe of the sexually mature Pelobates (periodic acid-Schiff reaction, magnification 1200 ×); i) formation of small droplets of PAS-positive substance in the cytoplasm of the intermediate lobe cells of the tadpole of the toad before metamorphosis (magnification 1200 ×).

bands from the intermediate lobe (see figure, a, a'). In these conditions, however, a mass of bordering capillaries can be seen. In the pond frog, for instance, attention was drawn to the regular geometrical orientation of the blood vessels, especially the bordering capillaries, buried equally in the parenchyma of the two lobes (see figure, b). In individual specimens of the lake frog, the bordering vessels formed wide lacunae buried in the superficial layers of the intermediate lobe (see figure, e).

Our findings were mainly obtained in adult specimens during multiplication. After hibernation the neurohypophysis and, in particular, the posterior lobe are enlarged in volume, hyperemic, and rich in granules of hypothalamic neurosecretion both in the species beginning to multiply immediately (pelobates and the toad) and in the species in which high sexual activity began much later (pond and lake frogs). With the onset of the period of multiplication, the intermediate lobe also enlarged.

In frogs the intermediate lobe was characterized by a compact arrangement of its constituent cells. On the other hand, in the toad the cells of the intermediate lobe are comparatively loosely arranged and form a distinctive syncytium. The cells of the intermediate lobe have much in common with the delta-basophils of the anterior lobe, and they are typically small in size, with a compact rim of homogeneous or granular cytoplasm and spherical nuclei.

After hibernation the cytoplasm was weakly basophilic and gave an obviously positive PAS reaction. When stained with paraldehyde-fuchsin, each cell showed the presence of 1-3 granules in the form of inclusions, staining rather more weakly than the granules of the basophilic cells of the anterior lobe. The cells of the anterior lobe of the hypophysis are usually full of such granules before the beginning of reproduction. During and after multiplication these granules were no longer observed in the cytoplasm of the elongated cells of the intermediate lobe.

The different cells of the intermediate lobe showed different staining properties of their karyoplasm: basophilia and well marked oxyphilia, becoming more intense after the emergence of the animals from the state of hibernation. The difference in staining of the nuclei of the different cells was intensified during the period of multiplication, and showed up more clearly after staining with chromic hematoxylin by Gomori's method with phloxine. The karyoplasm showed a sharp rise in the number of phloxinophilic structures, resembling the nucleolus in shape, which were responsible for the oxyphilic staining of the nucleus. There was a range of transitional forms between the ordinary nucleus, with its one or two nucleoli, and the nucleus filled with phloxinophilic inclusions. Phloxinophilic formations of the nucleolar type, like the nucleolus itself, could migrate into the cytoplasm, after which the nucleus, having lost its oxyphilic inclusions, became basophilic. In Pelobates the deformation of the nucleus after migration of the oxyphilic substance was especially marked. The tonus of the nuclear membrane was modified and its surface became uneven. The nucleus acquired an irregular polygonal shape. At the end of the period of multiplication nuclei of this type became predominant in the cells of the intermediate lobe. When stained with methyl green and pyronine, their nucleolus showed up very clearly.

Our findings in respect to karyocrinia in the cells of the intermediate lobe of the hypophysis in amphibians agree with the observations of Metuzals [11], who observed a similar phenomenon in the grass frog. However, the conclusions of this worker that the hypophysis of many frogs does not contain droplets of colloid requires some correction, for we found such formations in five different species. According to our findings, oxyphilic droplets were absent only in the intermediate lobe of animals during sexual inactivity.

In addition to the aldehyde-fuchsinophilic granules, the cytoplasm of the intermediate lobe cells, when stained by Gomori's method, was seen to contain very small phloxinophilic droplets, which stained orange by Halmi's method and gave a positive PAS reaction for polysaccharides. Usually one or two such droplets could be seen in different places in the cytoplasm, rarely several were seen lying close to the nucleus. Larger droplets were situated outside the cells, intercellularly. The appearance of intercellular droplets coincided with marked hyperemia of the vessels in the posterior lobe and at its border with the intermediate lobe. The tinctorial properties of the intercellular droplets and of the small droplets lying in the cytoplasm of the cells were identical. The intercellular droplets were phloxinophilic, stained with orange and Schiff's reagent, gave a positive reaction for RNA, and never took up aldehyde-fuchsin, differing in this respect from the granules of neurosecretion. Handa and Humamoto [7] stated that the phloxinophilic colloid of the intermediate lobe differs from the droplets of neurosecretory substance of the preoptic nuclei and the posterior lobe of the hypophysis by the absence of a phospholipid fraction. The number of these droplets of neurosecretory substance of the preoptic nuclei and the posterior lobe of the hypophysis by the absence of a phospholipid fraction. The number of these droplets reaches a maximum (45-50 droplets in one section) at the beginning of the period of multiplication, after which it falls sharply [1-5]. By fusion with each other, the droplets

may attain a large size. They are situated in different parts of the organ, but are concentrated above all close to the walls of the bordering vessels (see figure, s, d, e, f, g).

With the onset of high sexual activity in the cells of the intermediate lobe of the hypophysis, their content of nucleoproteins and polysaccharides increased (see figure, h), although at this time the cytoplasm of many cells became intensively vacuolated. Later, after the warning of sexual activity, the borders of the cytoplasm became ill-defined, and it began to appear hyalinized, containing no intracellular droplets. The intensity of staining for polysaccharides and RNA fell slightly by comparison with its intensity during the period preceding multiplication and at its beginning.

Our comparison of the microscopic pictures of the intermediate lobe of the hypophysis in the larvae of the various species showed that signs of activation of the intermediate lobe correspond in time to the period of the metamorphosis. These signs consisted of differentiation of the cells into two categories, differing in the tinctorial properties of the nuclei, the degree of swelling and increase in the chromophilia of the cytoplasm and the absence or presence of oxyphilic droplets in the cytoplasm, giving a positive PAS reaction (see figure, i). These droplets appeared before metamorphosis in the larvae of the green toad, in which the transformation took place soonest and at the most rapid rate. With the onset of metamorphosis the number of intercellular oxyphilic droplets in the larvae of all species rose. These droplets were most numerous next to the border with the posterior lobe and with the neural part of the hypophyseal stalk. The cytoplasm began to be filled at this time with a dust-like material, staining greenish-grey by Halmi's method. After metamorphosis the cytoplasm became homogeneous and stained more intensively with light green; oxyphilic droplets were seen extremely rarely.

No firm conclusion should be drawn concerning the physiological importance of the droplets of oxyphilic material in the intermediate lobe of the hypophysis, but it is evident that their appearance must bear some relation to the influence of the posterior lobe of the hypophysis, the principal depot of hypothalamic neurosecretion. The functional changes in the neurohypophysis may be judged by its content of neurosecretion and by its hyperemia [6, 8]. The appearance of droplets of neurosecretion coincides with the state of high functional intensity of the posterior lobe, as observed in periods of metamorphosis and multiplication.

In special experiments on the larvae of the various species we removed the region of the preoptic nuclei of the diencephalon, i.e., we excluded the source of hypothalamic neurosecretion. As a result of this operation hypertrophy of the intermediate lobe soon developed, its nuclei became slightly smaller, and it stained uniformly. All the tadpoles were usually black in color and failed to undergo metamorphosis [1]. This fact indicated absence of stimulation from the anterior lobe of the hypophysis. In the triton, according to Mazzi [10], after division of the base of the infundibulum and insertion of a celloidin disk into the line of section, the intermediate lobe hypertrophied and the basophilia of its cells increased.

The facts we have considered above demonstrate convincingly that the hypothalamic neurosecretion is essential for the formation of oxyphilic droplets in the intermediate lobe, and that the presence of these droplets is in no way associated with the formation of melanophorin. These droplets can also be seen in the basal areas of the median eminence and immediately adjacent to the walls of its dilated blood vessels. The formation of oxyphilic droplets in the the intermediate lobe of the hypophysis is clearly stimulated at times of increased function of the neurohypophysis during periods of biological processes differing widely in character (metamorphosis and multiplication), which are accompanied by increased function of the basophilic cells of the anterior lobe of the hypophysis. Hyperpigmentation of the skin is observed both in tadpoles undergoing operation and in some specimens found in natural conditions without both eyes, and caught among a batch of normal larvae. In these latter, in contrast to the tadpoles undergoing operation, oxyphilic droplets are present in the intermediate lobe of the hypophysis.

The formation of large numbers of oxyphilic droplets in the intermediate lobe should not, therefore, be related to the secretion of intermedin. The changes in the skin pigmentation during metamorphosis or multiplication are not very considerable in themselves, so that this factor cannot account for the considerable changes in the microscopic picture of the intermediate lobe. At the same time we cannot ignore the fact that the region of the anterior lobe of the hypophysis where it borders the intermediate lobe contains a preponderance of oxyphilic cells, the number and the zone of localization of which represent an adequate projection of the overlying intermediate lobe. The zonal nature of the distribution of oxyphilic and basophilic cells in the anterior lobe of the hypophysis, together with its abundant supply of blood enriched with neurosecretion, are matters requiring special examination and further explanation.

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