

## EXPERIMENTAL BIOLOGY

### INVERSION OF DIFFERENTIATION OF CELLS OF THE PARS INTERMEDIA OF THE PITUITARY

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Two frogs (*Rana ridibunda*), in a state of anabiosis, were subjected to whole-body  $\gamma$ -ray irradiation in a dose of 30,000 R. Examination of the pituitary revealed differentiation of cells of the pars intermedia into chromophil cells of the anterior lobe. This phenomenon is associated with the fact that a vessel from the anterior lobe penetrated into the pars intermedia, along which differentiation of typical chromophil cells mainly took place.

An essential factor in the development and organization of the pars intermedia of the pituitary is the presence of direct contacts between glandular cells and endings of axons of hypothalamic neurons. Fibers entering the parenchyma of the pars intermedia from the proximal part of the neurohypophysis terminate directly on the outer membranes of glandular cells ("neuroglandular synapses") [4, 5, 7, 11, 13]. Vascularization of the pars intermedia is slight in degree. In the amphibian pituitary large capillaries are located regularly at the boundary between the pars intermedia and posterior lobe, while in mammals they pass along connective-tissue septa dividing the glandular parenchyma into areas, none of which possesses its own blood supply. Conversely, in contrast to the pars intermedia, the parenchyma of the anterior lobe has direct hypothalamic innervation [8, 9], but it possesses an extremely well-developed system of vascularization. Most of the blood is carried by portal vessels into the anterior lobe from the proximal part of the neurohypophysis. The importance of hypothalamic neurohumors brought by the blood stream for the trophic functions of the anterior lobe is well known. The neurosecretory system thus induces morpho-functional differentiation of the adenohypophysis in various ways. Consequently, the factors regulating the pars intermedia and anterior lobe are not identical.

Further confirmation of this view is given by the phenomenon, discovered by the writers, which suggests that inversion of differentiation of cells in the pars intermedia can take place depending on whether the hypothalamus acts on it through nervous or vascular mechanisms.

#### EXPERIMENTAL METHOD

Whole-body irradiation with  $\gamma$  rays in a dose of 30,000 R (dose rate 55 R/sec) was given to 73 frogs (*Rana ridibunda*) in a state of anabiosis, and the animals were divided into two groups. The animals of group 1 were left in the state of anabiosis at a temperature of 4°C, while those of group 2 were kept at 25°. Unirradiated frogs (group 3) acted as the control. Massive death of the animals of group 2 was observed 7 days after irradiation, while the animals of group 1, kept in a state of anabiosis after irradiation, were sacrificed 15 days later with no evidence of lethal disease. Some animals were allowed to survive for a longer period and were decapitated after 17 days. The pituitary glands of the frogs, together with the brain, were fixed in Bouin's fluid and stained with Gomori's paraldehyde-fuchsin, and counterstained by Halmi's

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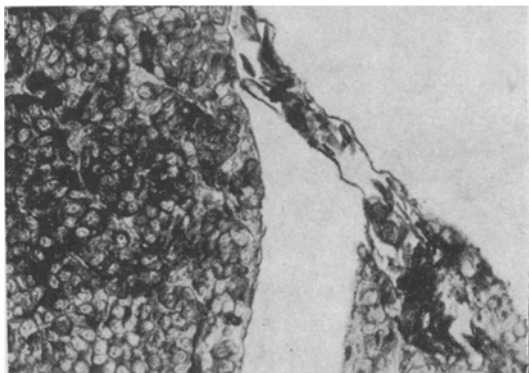


Fig. 1. Vessel connecting the anterior lobe and pars intermedia. Paraldehyde-fuchsin (Gomori-Gabe), 200  $\times$ .

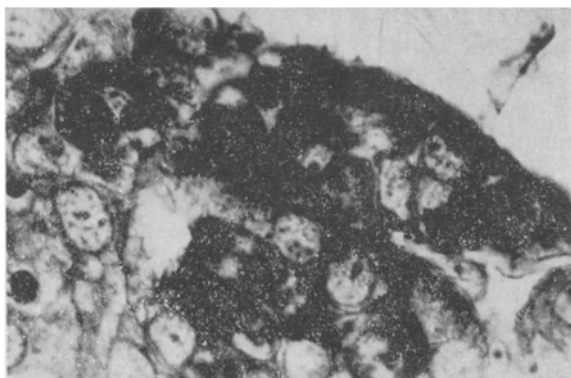


Fig. 2. Basophil cells, homologous with basophils of anterior lobe, make contact in parenchyma of pars intermedia with capillary shown in Fig. 1. Halmi, 720  $\times$ .

PF- and PAS-positive basophils were localized around the vessels growing into the pars intermedia, while oxyphils were represented by only two or three cells. The nuclei of individual chromophil cells contained several accessory nucleoli, indicating their increased functional activity.

A similar phenomenon under other conditions was obtained experimentally in *Bufo arenarum* [12] after removal of the anterior lobe of the pituitary; 60 days after this operation, the presence of capillaries was found in the pars intermedia, which must have come from the median eminence of the neurohypophysis. Along the endothelium of these newly formed vessels were located typical basophils and oxyphils characteristic of the anterior lobe of the pituitary. The formation of chromophil cells in the parenchyma of the pars intermedia in Rodrigues's experiments correlates with the appearance of neurosecretion in the median eminence.

This phenomenon deserves special attention, because differentiation of chromophil cells in the pituitary depends on the action of a neurosecretory substance brought from the hypothalamus by the blood of the portal capillaries [1, 3, 10].

On the other hand, nervous connections are important for differentiation of the cells of the pars intermedia, and their interruption experimentally deprives this part of the gland of its usual functional correlations, leading to unusual hypersecretion of its cells [2, 7].

Hence, differences in the nature of the regulatory factors and in the methods of their influence on the glandular parenchyma determine the specific features of differentiation of the pathological picture and of

method and with Schiff's reagent. The trichrome-PAS reaction was also carried out at the same time, thereby enabling the thyrotrophs and gonadotrophs in the pituitary of the frogs to be distinguished by their staining properties [6].

## EXPERIMENTAL RESULTS

In two frogs, in a state of winter anabiosis and subjected to whole-body  $\gamma$ -ray irradiation in a dose of 30,000 R, the vascularization of the pars intermedia was increased, and single capillaries, which were not observed in other animals, appeared in the parenchyma of the gland. In sagittal histological sections through the pituitary, a capillary could be seen running from the neurohypophysis into the region of the pars intermedia bounding the anterior lobe. This vessel was dilated and filled with erythrocytes (Fig. 1). Around this unusual capillary in the pars intermedia of the pituitary, on its endothelial cells, basophils and oxyphils could be seen, identical in their structure and staining properties with cells of the anterior lobe of the pituitary (Fig. 2).

The pars intermedia of the pituitary of intact animals in a state of anabiosis contains round cells of uniform type, with cytoplasm packed with fine PAS-positive and paraldehyde-fuchsinophilic (PF) granules, with varied staining activity. By means of the trichrome-PAS staining method, and using the classification of Cordier and Herlant, typical gonadotrophs (brick-red cells) and thyrotrophs (crimson cells) could be detected among the basophilic cells in the anterior lobe of the pituitary.

The cytoplasm of cells of the pars intermedia in direct contact with the wall of the vessel running from the anterior lobe into the pars intermedia, like basophils of the anterior lobe, was filled with large glycoprotein and PF granules. After staining with trichrome-PAS, the newly formed basophils of the pars intermedia were stained crimson only, like the thyrotrophs of the anterior lobe. Mainly

hormonopoiesis in the adenohypophysis. Differences between the connections of different parts of the adenohypophysis with the sources of neurosecretory substance are evidently determined phylogenetically. This substance reaches the pars intermedia along axons, while it reaches the anterior lobe entirely via the vascular system.

It can be assumed that the blood vessel going into the pars intermedia drags after it the epithelial cells of the parenchyma of the pars tuberalis or anterior lobe. Such a phenomenon is actually seen in the case of proliferation of epithelium penetrating into intervacular spaces. However, this assumption is no argument against the possibility of differentiation of parenchyma in the pars intermedia in the same manner as in the anterior lobe after the change in character of the regulatory influences. There is no question of the reliability of the results of Rodrigues's experiments [12] involving adenohypophysectomy. This worker removed the anterior lobe of a frog without injuring the capsule, so that the possibility of spreading of epithelial cells of the anterior lobe into the pars intermedia was completely ruled out. However, cells identical with the basophils and oxyphils of the anterior lobe were formed in the pars intermedia. Rodrigues correctly explains this phenomenon by the occurrence of vascularization. After establishment of vascular connections, the direction of differentiation of glandular cells in the pars intermedia was radically changed, and took place in the same way as in the anterior lobe. Consequently, there are grounds for the general conclusion that the humoral or nervous connection with the hypothalamus and neurohypophysis determines the formation of morpho-functional characteristics and properties of different parts of the adenohypophysis.

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