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PRELIMINARY REPORT ON THE EFFECT OF PITUITARY AUTOTRANSPLANTATION ON THE PARS INTERMEDIA OF THE SEAWATER GOBY, *GILLICHTHYS MIRABILIS* [1]

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RELATO PRELIMINAR SOBRE OS EFEITOS DO AUTOTRANSPLANTE DA PITUITÁRIA NA PARTE INTERMÉDIA DO CADOZ DE ÁGUA SALGADA, *GILLICHTHYS MIRABILIS*

RESUMO

Estudou-se com a microscopia eletrônica os efeitos do autotransplante da parte intermédia da pituitária do cadoz de água salgada, *Gillichthys mirabilis.*

No peixe sem transplante, a parte intermédia possui dois tipos de células secretoras: hematoxilina de Pb-positiva (PbH+) e hematoxilina de chumbo negativa (PbH−), diretamente inervadas pelos axônios neurossecretores (tipo A e B) originários do hipotálamo.

Após o autotransplante, a parte intermédia revasculariza-se rapidamente e uma semana após o transplante as células PbH+ possuem pequeno número de grânulos secretores, retículo endoplasmático tortuoso e bem desenvolvido.

Depois de quatro semanas as células PbH+ aparecem bem ativas e após quatro meses muitas vesículas secretoras acumularam-se nas células, embora algumas ainda possuam muito retículo endoplasmático tortuoso, como nos peixes controles.

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Número especial em homenagem ao Prof. Dr. Paulo Sawaya, no ano jubilar de seu magistério.

As células PbH− respondem de forma similar. É possível que o controle hipotalâmico sobre a parte intermédia é restabelecido via circulação sistêmica.

ABSTRACT

The effect of autotransplantation of the pars intermedia of the pituitary of the seawater goby, *Gillichthys mirabilis,* was studied by electron microscopy. In intact fish, the pars intermedia contains two types of secretory cells: lead hematoxylin-positive (PbH+) and lead hematoxylin-negative (PbH−) cells, directly innervated by neurosecretory axons (types A and B) from the hypothalamus. After autotransplantation, the pars intermedia was revascularized rapidly. One week after transplantation, the PbH+ cells contained small numbers of secretory granules and well-developed rough endoplasmic reticulum. After four weeks, the PbH+ cells appeared very active; after four months many secretory vesicles had accumulated within the cells, although some cells still contained much rough endoplasmic reticulum, as in intact fish. The PbH− cells responded similarly. It is possible that hypothalamic control over the pars intermedia is reestablished via the systemic circulation.

INTRODUCTION

The pars intermedia of the teleost pituitary is composed of two distinct secretory cell types: one stains with lead hematoxylin (PbH^+ cells) and the other does not stain with this dye but does react with the periodic acid Schiff (PAS) method and are referred to herein as PbH^- cells (*cf.* Ball and Baker, 1969; Sage and Bern, 1972). The PbH^+ cells appear to secrete MSH, whereas the PbH^- cells have an uncertain function, possibly related to osmoregulation (Olivereau and Ball, 1970). These two cell types are directly innervated by two kinds of neurosecretory fibers: types "A" and "B" The nature of the regulation accomplished by these axons is of obvious interest. Recently, some morphological studies have begun to elucidate this problem (Zambrano *et al.,* 1972; Follenius, 1972), and the hypothalamic control of the pars intermedia in teleost fishes has been recently reviewed (Ball *et al.,* 1972).

Some light-microscope studies have been carried out on the pars intermedia transplanted away from the hypothalamus (*Poecilia formosa* by Ball *et al.,* 1965, and by Olivereau and Ball, 1966; *Poecilia latipinna* and *Anguilla anguilla* by Olivereau, 1969; *Gasterosteus aculeatus* by Leatherland, 1970). However, the information accumulated to date is insufficient to provide a complete understanding of hypothalamic control of the teleost pars intermedia. The effect of transplantation on the rostral pars distalis of the *Gillichthys* pituitary has been reported (Nagahama *et al.*, 1973). Incidental to that study, we decided to examine the effects of autotransplantation on the cells of the pars intermedia.

MATERIALS AND METHODS

Gillichthys mirabilis, a gobiid teleost species from San Francisco Bay, were held in seawater tanks at 14°C and fed on live brine shrimp. All fish were acclimated for a minimum of one week before autotransplantation. The intact pituitary from anesthetized fish was carefully removed and transplanted into a prepared cavity under the tongue (Nagahama *et al.*, 1973). In this position the grafted pituitary is clearly visible through the oral mucosa and the course of revascularization, which takes about a week, can be followed.

A total of 7 controls and 14 autotransplanted fishes were examined by electron microscopy. Eight fishes were killed after 1-2 weeks, four fishes after 4 weeks and two fishes after 4 months of auto-transplantation. The pituitary was removed and initially placed in a modified glutaraldehyde/paraformaldehyde fixative. Cacodylate-buffered osmium tetroxide was used for post-fixation, and the tissues were dehydrated in graded ethanols and embedded in epoxy resin (Spurr, 1969). Thick sections (1 μ) were cut and stained with methylene blue-azure II (Richardson, *et al.*, 1961). Thin sections were cut with glass and diamond knives and studied in a Siemens Elmskop II or Phillips 200 electron microscope.

RESULTS

Intact fish. The intact pituitary is located within a depression in the tuberal region of the hypothalamus of *Gillichthys*. Neurohypophysial tissue stainable with aldehyde fuchsin covers the outer surface of the posterior part of the pituitary which comprises the pars intermedia (Fig. 1). Neurohypophysial processes also penetrate among the cells of the pars intermedia. Of the two cell types in the pars intermedia, the lead hematoxylin-positive type (PbH^+) is more numerous. The other cell type does not stain with lead hematoxylin (PbH^-).

In 1 μ-thick epoxy sections stained with methylene blue-azure, the overall distribution of the pars intermedia cells as well as the ramifications of the neurohypophysial tissue could be clearly observed (Fig. 1).

Ultrastructurally, numerous axons filled with type A granules are found at the periphery of the pars intermedia. Type A axons are also found among the pars intermedia cells, and some axons are indented deeply into the cells. In addition, type B axons, less numerous and smaller in size, are also found among the pars intermedia cells (Fig. 4).

The major cell type (PbH^+) contain large pale granular vesicles, filling the cytoplasm of most of the cells along with some rough endoplasmic reticulum (Fig. 4). A small proportion of these cells in control fish exhibit multilayered endoplasmic reticulum.

The second cell type (PbH^-) contain dense spherical granules which fill most of the cytoplasm (Fig. 4). Based on ultrastructural criteria, these cells show low to moderate activity. Their processes are narrow and tenuous, superficially resembling type A axons.

One to two weeks after autotransplantation

One to two weeks after autotransplantation, the neurohypophysial tissue shows variable signs of disintegration (Fig. 2). Most of the axons appear within vacuoles of the phagocytes which apparently invade the transplanted pituitary (Fig. 5).

Most PbH^+ cells show extensive development of rough endoplasmic reticulum, which takes the form of concentric whorls as well as other multilayered arrangements (Fig. 5). These highly active-appearing cells contain relatively few secretory vesicles. These is an indication of greater development of the endoplasmic reticulum in the vicinity of massive neurohypophysial disintegration. However, a few PbH^+ cells contain many secretion granules essentially similar to those found in control pituitaries.

The PbH^- cells appeared more active and contained more endoplasmic reticulum than in the control pituitaries, and they contained fewer dense granules (Fig. 5).

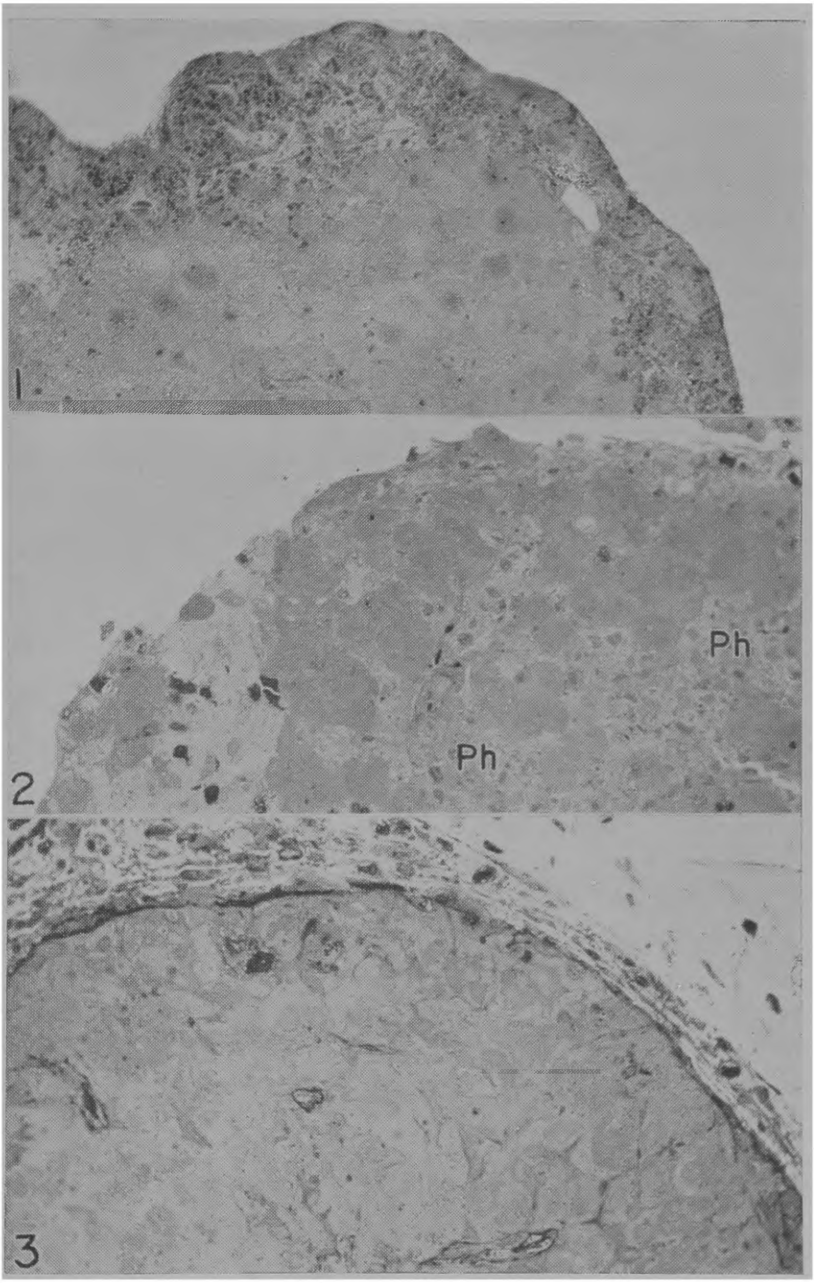

Fig. 1 — Cross section of pars intermedia of seawater *Gillichthys*. Neurohypophysis forms distinctive layer at periphery. Secretory cells are pale and cellular borders are indistinct. 1 μ methylene blue-azure. 400 ×.

Fig. 2 — Cross section of pars intermedia of 2 week autotransplanted seawater fish. Note absence of neurohypophysial tissue and invasion of phagocytes (Ph) among darker staining secretory cells. 1 μ methylene blue-azure. 400 ×.

Fig. 3 — Cross-section of pars intermedia of 4 month autotransplanted seawater fish. Note presence of phagocytes (Ph) in connective tissue at periphery but absence from interior of pars intermedia. Secretory cells appear pale as in control (Fig. 1). 1 μ methylene blue-azure. 400 ×.

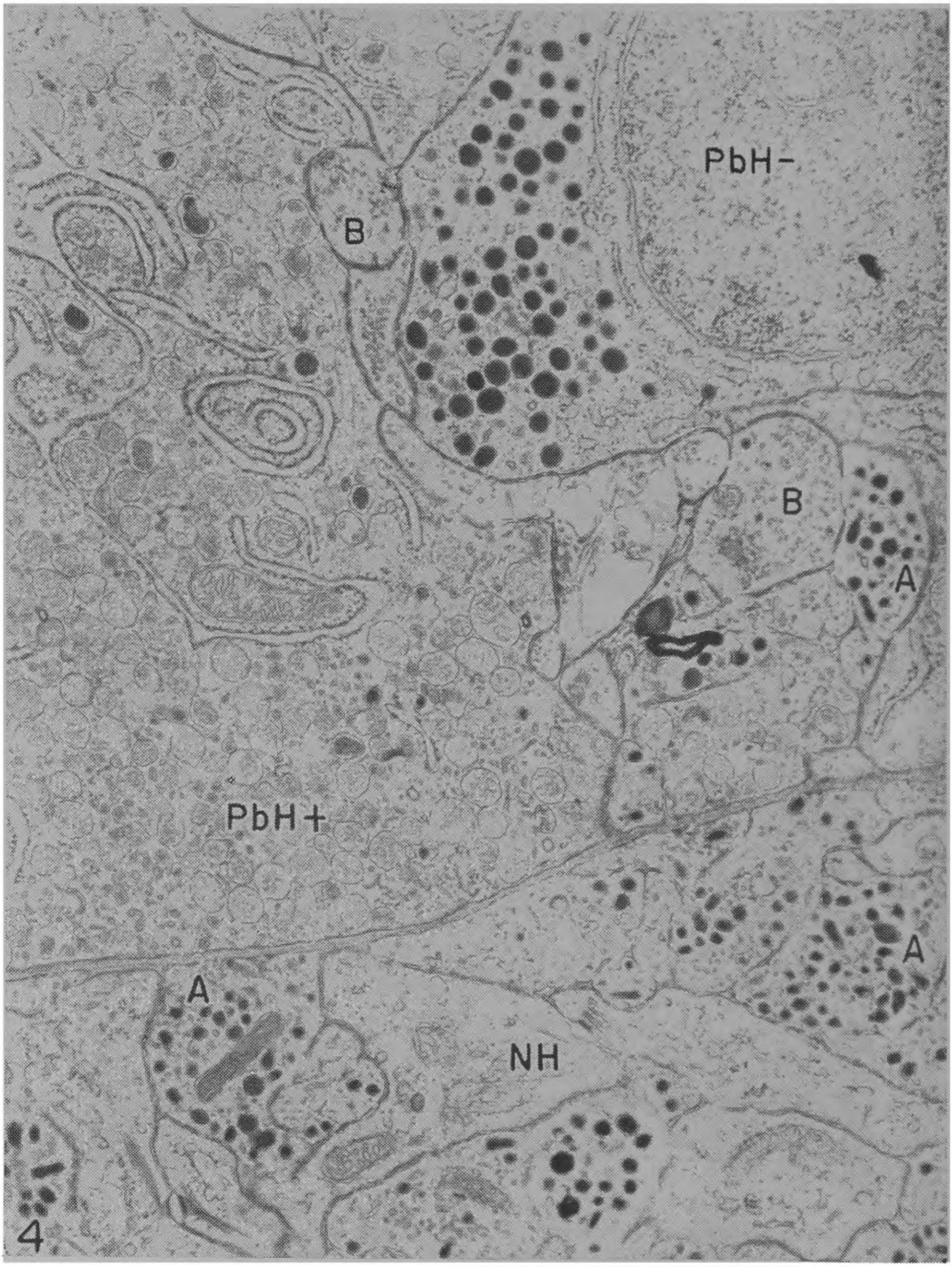

Fig. 4 — Electron micrograph of seawater *Gillichthys* pars intermedia. Portions of PbH− and PbH+ cells are shown. Note types A and B neurosecretory axons from neurohypophysis (NH). 20,000 ×.

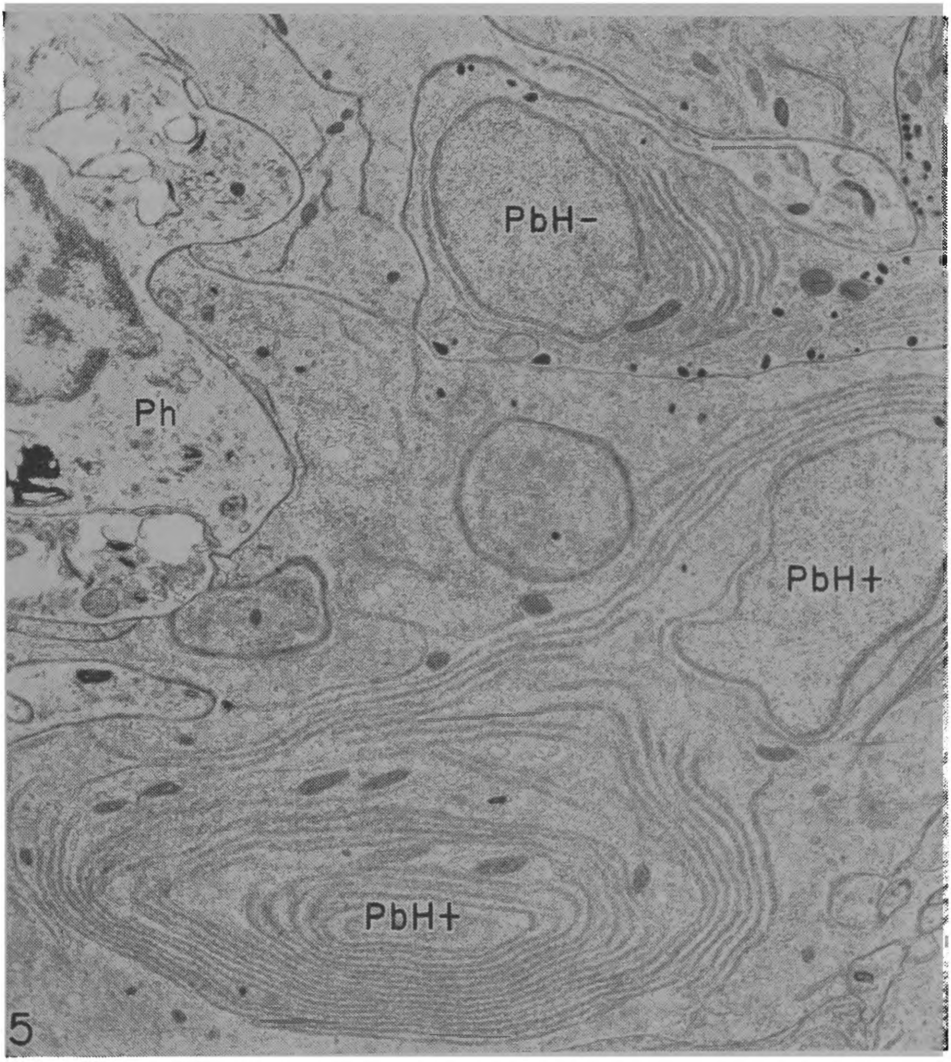

Fig. 5 — Electron micrograph of seawater *Gillichthys* pars intermedia 2 weeks after autotransplantation. Note extensive endoplasmic reticulum in PbH+ cell. Phagocyte (Ph) contains neurohypophysial debris. PbH− cell contains dense granules and some endoplasmic reticulum. 8,000 ×.

Four weeks after autotransplantation

The pars intermedia of pituitaries transplanted for four weeks maintains a high degree of cellular activity as indicated by the quantity of rough endoplasmic reticulum. The size of some of the concentric whorls is somewhat diminished, but there appear to be more of them. Only a few phagocytes occur among the granular cells, although many are still present at the periphery of the transplant. A few of the PbH^+ cells are very osmiophilic and appear to be undergoing degeneration. The PbH^- cells also show undiminished cellular activity.

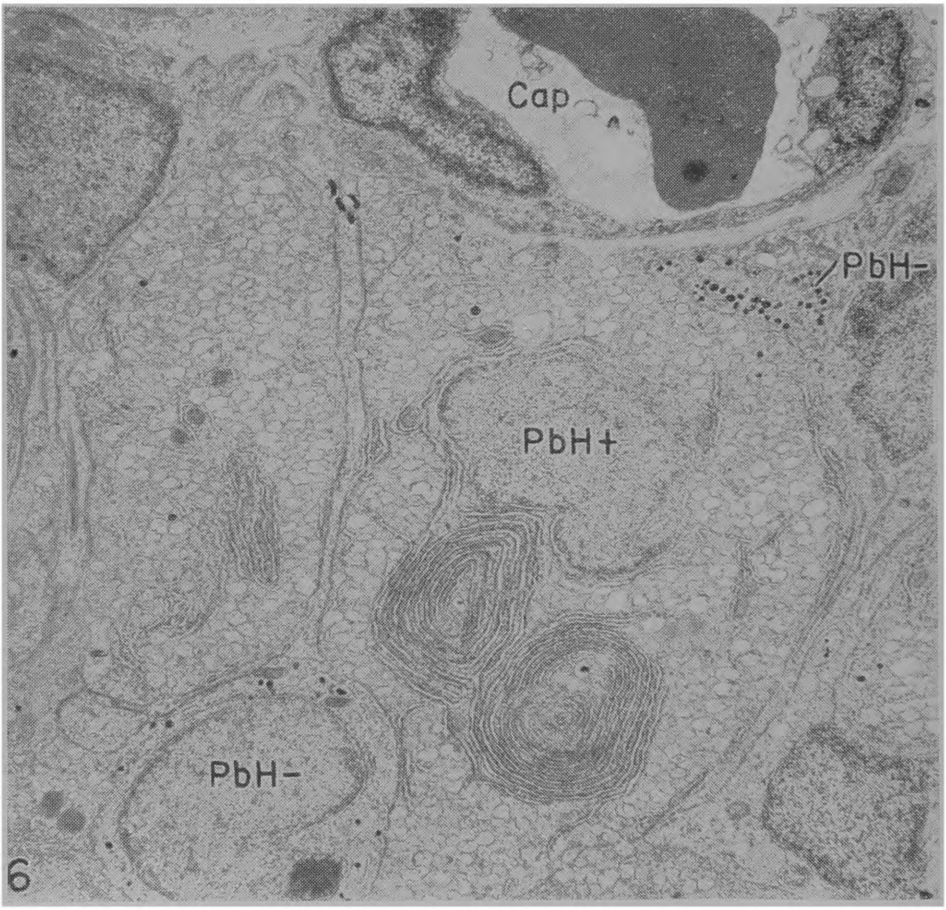

Fig. 6 — Electron micrograph of seawater *Gillichthys* pars intermedia 4 months after autotransplantation. Note dilated secretory vesicles in PbH+ cell near capillary (Cap). Small PbH− cell and process are present. 8,000×.

Four months after autotransplantation

Four months after transplantation, very few phagocytes are seen within the pars intermedia, although many are still present around the capsule (Fig. 3). Many PbH^+ cells are large and full of secretory vesicles and appear superficially similar to control cells. On closer examination, however, these secretory vesicles are larger and contain less granular material (Fig. 6). Other cells also show an extensive endoplasmic reticulum. A few large osmiophilic inclusions are evident in some PbH^+ cells. In 1 μ sections many of the secretory cells appeared larger than in the controls. Most of the PbH^- cells contain many large dense secretion granules. When present, the rough endoplasmic reticulum forms layers around the nucleus. These cells possess tortuous processes which occupied some of the interstitial space between PbH^+ cells (Fig. 6). Some smaller cells contain fewer granules, but otherwise appear active.

DISCUSSION

The pars intermedia in *Gillichthys mirabilis* as in other teleosts contains two cell types, one of which is lead-hematoxylin-positive (PbH^+) and the other is either periodic acid Schiff-positive or lead hematoxylin-negative (PbH^-), depending on the species (*cf*. Ball and Baker, 1969; Sage and Bern, 1972). Electron microscope studies show that types A and B axons make direct contact with both cell types. In intact fish both cell types are presumably controlled to a major degree by these axons. When the physical connection with the hypothalamus is lost following pituitary transplantation, both axonal types completely disintegrate within two weeks, and the transplanted pituitary cells are consequently without direct hypothalamic control.

The notable hypertrophy of the rough endoplasmic reticulum of PbH^+ cells one to two weeks after autotransplantation suggests lifting of inhibition, ascribable to the disintegration of the aminergic type B fibers; similar changes occur after 6-hydroxydopamine treatment which selectively destroys type B fibers (Zambrano *et al.*, 1972). The effect of type A axon degeneration is unknown. In contrast to *Gillichthys,* in *Poecilia formosa* homotransplants, the PbH^+ cells became atrophied and scarce (Olivereau and Ball, 1966). One to two weeks after homotransplantation in *Gasterosteus aculeatus,* the grafted chromophobes (possibly equivalent to our PbH^+ cells) became significantly smaller than normal (Leatherland, 1970). On the other hand, in *P. latipinna,* autotransplants are well maintained at least for 3 weeks and show moderate activity (Olivereau, 1969). Thus, the response of the PbH+ cells after pituitary transplantation appears to vary with the species studied.

Many PbH+ cells in the transplants showed a considerable number of secretory vesicles after four months, resembling to some degree the condition which exists in intact fish. Maintenance of PbH+ cells in autotransplants after two months has also been reported in *Anguilla* (Olivereau, 1969). The possibility of distant control of the pars intermedia by the hypothalamus cannot be discounted, although direct evidence is lacking as yet. Inasmuch as the axonal processes of the preoptic neurons (type A fibers) and the lateral tuberal neurons (type B fibers) were transected during hypophysectomy prior to transplantation, formation of a neurohemal structure by the proximal axon stumps is highly possible (Sathyanesan, 1965). Control of the ectopic pituitary could then be achieved via the systemic circulation.

There are relatively few PbH- (electron-dense granule-containing) cells in intact *Gillichthys.* These cells were also found four months after autotransplantation. Crude estimates based on 1 μ sections and on electron micrographs indicate that there was no decrease in the number of these cells. Therefore, it is possible that neither stimulatory nor inhibitory factors directly control the functional activity of these cells. Our observations differ from those on *Poecilia formosa* (Olivereau and Ball, 1966), *P. latipinna* (Oliveireau, 1969) and *Gasterosteus* (Leatherland, 1970) in that the *Gillichthys* cells remain active.

This preliminary study opens avenues for further investigation. The possibility of systemic control of the autotransplant by the hypothalamus via a regenerated neurohemal structure is raised. Functional significance of this new control system can be examined by challenging autotransplanted fish with light and dark backgrounds in order to see how rapidly the pars intermedia cells are able to respond. Further experiments are now in progress.

REFERENCES

BALL, J. N. and BAKER, B. I. (1969) — The pituitary gland: anatomy and histophysiology. *In* "Fish Physiology" (W. S. Hoar and D. J. Randall, eds.), Vol. II, pp. 1-110. Academic Press, New York and London.

BALL, J. N.; OLIVEREAU, M.; SLICKER. A. M. and KALLMAN, K. D. (1965) — Functional capacity of ectopic pituitary transplants in the teleost *Poecilia formosa,* with a comparative discussion on the transplanted pituitary. Phil. Trans. B **249**:69-99.

BALL, J. N.; BAKER, B. I.; OLIVEREAU, M. and PETER, R. E. (1972) — Investigations on hypothalamic control of adenohypophysial functions in teleost fishes. Gen. Comp. Endocrinol., Suppl. **3**:11-21.

FOLLENIUS, E. (1972) — Ultrastructure of the hypophysial aminergic nerves in the teleost *Gasterosteus aculeatus* degenerating after application of 6-hydroxydopamine. Z. Zellforsch., **128**:69-82.

LEATHERLAND, J. F. (1970) — Histological investigation of pituitary homotransplants in the marine form (trachurus) of the threespine stickleback, *Gasterosteus aculeatus* L. Z. Zellforsch. **104**:337-344.

NAGAHAMA, Y.; NISHIOKA, R. S. and BERN, H. A. (1973) — Structure and function of the transplanted pituitary in the seawater goby, *Gillichthys mirabilis.* I. The rostral pars distalis. (Submitted for publication).

OLIVEREAU, M. (1969) — Activité de la pars intermedia de l'hypophyse autotransplantie chez l'Anguille. Z. Zellforsch. **98**:74-87.

OLIVEREAU, M. and BALL, J. N. (1966) — Histological study of functional ectopic pituitary transplants in a teleost fish (*Poecilia formosa*). Proc. Roy. Soc. B **164**:106-129.

OLIVEREAU, M. and BALL, J. N. (1970) — Pituitary influences on osmoregulation in teleosts. Mem. Soc. Endocrinol. **18**:57-85.

RICHARDSON, K. C.; JARETT, L. and FINKE, E. H. (1961) — Embedding in epoxy resins for ultrathin sectioning in electron microscopy. Stain Technol. **35**:313-323.

SAGE, M. and BERN, H. A. (1972) — Cytophysiology of the teleost pituitary. International Review of Cytology **31**:339-376.

SATHYANESAN, A. G. (1965) — The reorganization of the hypophysial stalk following hypophysectomy in the teleost *Porichthys notatus* Girard. Z. Zellforsch. **67**:734-739.

SPURR, A. R. (1969) — A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res. **26**:31-43.

ZAMBRANO, D.; NISHIOKA, R. S. and BERN, H. A. (1972) — The innervation of the pituitary gland of teleost fishes. *In* "Brain-Endocrine Interaction. Median Eminence: Structure and Function. Int. Symp. Munich 1971", 50-66. Karger, Basel.