

HYPOPHYSIAL FINE STRUCTURE OF
THE CHANNEL CATFISH
ICTALURUS PUNCTATUS

A Thesis
Presented to
The Department of Biology
East Carolina University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science in Biology

by
Everett VanDeveer Davis, Jr.

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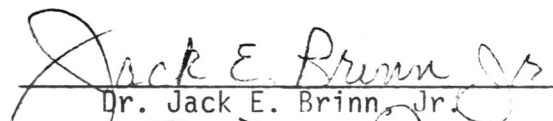
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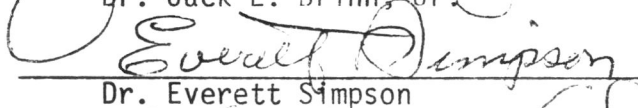
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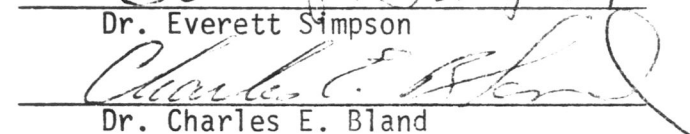
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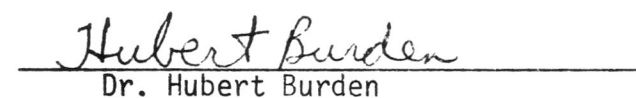
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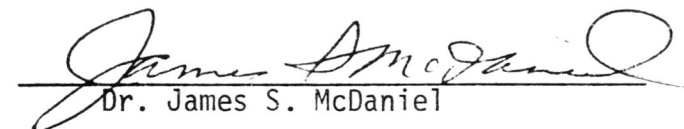
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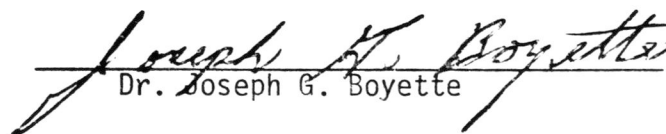

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ABSTRACT

Everett VanDerveer Davis, Jr. HYPOPHYSIAL FINE STRUCTURE OF THE CHANNEL CATFISH ICTALURUS PUNCTATUS. (Under the direction of Dr. Jack E. Brinn, Jr.) Department of Biology, April 1978.

The hypophysial fine structure of Ictalurus punctatus was described from light and electron microscopic observations. Presumptive prolactin, adrenocorticotrophic, and thyrotrophic cells are described in the rostral pars distalis; presumptive thyrotrophic, somatotrophic, and gonadotrophic cells are described in the caudal pars distalis; and presumptive melanocyte stimulating cells in addition to two other cell types are described in the neurointermediate lobe. Nerve fibers originating in the hypothalamus innervate the hypophysis with two types of neurosecretory fibers, type A and type B. These fibers form synaptic complexes with each of the presumptive endocrine cells. Type B fibers are found in the rostral pars distalis, caudal pars distalis and neurointermediate lobe, whereas the type A fibers are solely located in the neurointermediate lobe.

DEDICATION

This thesis is dedicated to my wife, Lynda and son, Tyler.
Their love and encouragement made the completion of this paper possible.

LIST OF FIGURES

	Page
Figure 1. Rostral pars distalis - Prolactin cell and TSH cell.	36
Figure 2. Rostral pars distalis - Prolactin cell.	38
Figure 3. Rostral pars distalis - ACTH cell	40
Figure 4. Caudal pars distalis - GTH cell and STH cell.	42
Figure 5. Caudal pars distalis - TSH cell	44
Figure 6. Caudal pars distalis - TSH cell and GTH cell.	46
Figure 7. Caudal pars distalis - TSH cell, STH cell, nerve tract	48
Figure 8. Neurointermediate lobe - PAS cell	50
Figure 9. Neurointermediate lobe - PbH cell	52
Figure 10. Neurointermediate lobe - cell type III.	54
Figure 11. Rostral pars distalis - Light staining chromophobe. . .	56
Figure 12. Rostral pars distalis - Dark staining chromophobe . . .	58

TABLE OF CONTENTS

	Page
LIST OF FIGURES	V
INTRODUCTION.	VI
LITERATURE OF REVIEW.	I
MATERIALS AND METHODS	15
RESULTS	
<u>Rostral Pars Distalis</u>	18
<u>Caudal Pars Distalis</u>	20
<u>Neurointermediate Lobe</u>	21
DISCUSSION.	24
FIGURES	35
BIBLIOGRAPHY.	58

INTRODUCTION

Recently, much research has been conducted on fish endocrines, particularly the pituitary gland. While this research has clarified many questions regarding the metabolism, reproduction, and osmoregulation in fish, it has also led to the posing of questions relating to the functional organization and mechanisms of control of endocrine functions. The histological organization of the fish pituitary is unique among vertebrates which not only facilitates the study of this gland but might also provide insight into the organization of other vertebrate pituitaries. Fish differ greatly from other vertebrates, and it is this unique difference in histological organization that facilitates pituitary studies. Teleost pituitary cells secreting a particular hormone are generally segregated into distinct regions of the gland thus allowing easy separation for observation and experimentation.

To date, the hormones secreted by the pituitary in many species have been isolated and characterized. Cell types have been described, vascular supplies traced, and control mechanisms studied. Although this work has been extensive in some teleosts, few studies have involved Ictaluridae. Therefore, the intent of this study is to describe the cell types found in the pituitary of the channel catfish, Ictalurus punctatus, according to their granular morphology and location within the gland. In addition, the innervation will be described with emphasis on nerve types, areas of innervation, and cell types innervated.

LITERATURE REVIEW

In order to fully understand the structure of the fish pituitary, a brief survey of the mammalian gland is in order. The mammalian pituitary is located in a depression of the sphenoid bone and is surrounded by the dura mater. The gland is attached to the infundibulum which is a projection of the hypothalamus. The adenohypophysis forms the anterior most portion of the gland and is composed of the pars distalis and the pars intermedia. The pars nervosa is the posterior-most portion of the gland and is comprised of nerve cell endings whose bodies lie in the paraventricular and supraoptic nuclei within the anterior and lateral portions of the hypothalamus.

The hormones of the pituitary are released by specific endocrine cells and transported to target cells or organs by the vascular system. Six protein or polypeptide hormones have been extracted from pars distalis of mammals and six cell types have been described, although it should be noted that it is possible for a single cell to secrete more than one hormone. (For this reason, a one to one relationship may not be correctly assumed in all cases.) Even though progress has been made in cytochemical techniques, it can not be concluded emphatically that any stain unmistakably identifies a particular hormone with its cell of origin. The six hormones isolated to date are growth hormone or STH, prolactin, adrenocorticotrophic hormone or ACTH, follicle stimulating hormone or FSH, luteinizing hormone or LH, and thyrotrophic stimulating hormone or TSH. The neurohormones oxytocin and vasopressin are produced within the neuron cell bodies in the hypothalamus and are transported down the axons for storage in swollen axon terminals termed Herring bodies in the neurohypophysis.

The neurohomones contained within the Herring bodies are discharged around blood vessels in the pars nervosa.

The teleost adenohypophysis has been described in the eel, Anguilla anguilla, (Knowles and Vollrath, 1966b), the molly, Poecilia latipinna, and P. formosa, (Olivereau and Ball, 1964; and Batten and Ball, 1975), the trout, Salmo gairdneri, (Olivereau, 1954), sockeye salmon, Oncorhynchus nerka, (Cook and Van Overbeeke, 1972), the striped mullet, Mugil cephalus, (Olivereau, 1968, and Abraham, 1970, 1973), the seahorse, Hippocampus sp. (De Lage, 1958), the goby, Gillichthys mirabilis, (Nagahama, et al., 1974), the goldfish, Carassius auratus, (Leatherland, Ball and Hyder, 1974), and the Indian catfish, Heteropneustes fossilis (Baker et al., 1974). Also numerous reviews are available on teleostian pituitary morphology (Pickford and Atz, 1957; Olivereau, 1954, 1963b; Stahl, 1963; Wingstrand, 1966; Ball and Baker, 1969; Sage and Bern, 1971; and Schreibman, Leatherland and McKeown, 1973).

The teleost gland is divided into four regions including the rostral pars distalis (RPD the caudal pars distalis (CPD), the more posterior pars intermedia (PI), and the neurohypophysis (NH) which interdigitates all regions of the gland (Ball and Baker, 1969).

Rostral Pars Distalis

The teleost RPD includes a large mass of carminophilic cells, presumably prolactin cells, interspersed with chromophobes, cells which take up little or no stain. Along the dorsal boundry of this region lies a border of variably staining cells, the presumptive adrenocorticotropic (ACTH) cells (Ball and Baker, 1969).

Prolactin cells found in Anguilla anguilla (Knowles and Vollrath, 1966b; Vollrath, 1966; Hopkins and Baker, 1968) and Conger conger (Knowles and Vollrath, 1966b) and in a number of salmonids, Oncorhynchus nerka (Van Overbeeke and McBride, 1967; Fagerland et al., 1968; Cook and Van Overbeeke, 1969; McBride and Van Overbeeke, 1969; Nagahama and Yamamoto, 1969a; McKeown and Van Overbeeke, 1971; Van Overbeeke and McBride, 1971; McKeown and Leatherland, 1973), O. keta, (Nagahama and Yamamoto, 1970) are elongated, pyramidal-shaped cells arranged in follicles and surrounded by a tri-laminar basement membrane (McKeown and Leatherland, 1973). The basement membrane stains with periodic-acid Schiff (PAS) and is thought to actually be an inter-vascular channel which expands to encompass capillaries or the outer surfaces of neural processes (Knowles and Vollrath, 1966a, 1966b, Leatherland et al., 1966; Leatherland, 1967; Follenius and Porte, 1962).

Olivereau and Herlant (1960) reported the similarity in the staining properties of the teleost carminophilic cell and the mammalian prolactin cell. Experimentation with the carminophilic cells of the RPD shows that they are the source of prolactin and that this hormone functions in osmoregulation in many species investigated (Ball, 1969a,b; Ensor and Ball, 1972).

Hypophysectomized freshwater and euryhaline fish (Schreibman and Kallman, 1966; Ball and Baker, 1969; Chidambaram, Meyer and Hasler, 1972) will die in a short period of time when placed in fresh water. Furthermore, fish treated with drugs like reserpine or 6-hydroxydopamine that interfere with prolactin release by depleting the adrenergic transmitters or destroying

the nerve terminals (McKeown, 1972; Zambrano et al., 1973) die when placed in fresh water (Sage and Bern, 1971). Administration of either L-dopa or 2-Br- α -ergocryptine to freshwater eels reduced normal granule loss in prolactin cells (Olivereau, 1975). Fish prolactin evidently plays an important role in salt and water metabolism by limiting the outflux of sodium ions from the body (Ensor and Ball, 1968, 1969a; Lahlou and Sawyer, 1969).

Adrenocorticotrophic (ACTH) cells have been identified in many fish and experimentation has shown their effect on interrenal activity and osmoregulation (Ball and Baker, 1969; Olivereau and Ball, 1970; Sage and Bern, 1971). Their typical position posterior to the prolactin cells in the RPD facilitates their identification. In the sockeye salmon, for example, the ACTH cells have been described as a "well defined group" in the dorsorostral area of the caudal pars distalis (Van Overbeeke and McBride, 1967). These cells are generally polymorphic or columnar forming a wedge of cells at the interface of the RPD and the neurohypophysis. The neurohypophysis is separated from the ACTH cells by a PAS-positive basement membrane (Knowles and Vollrath, 1966a; Abraham, 1970; Leatherland, 1972; Baker et al., 1973). The staining properties of ACTH cells vary, but in most species they are delineated with the lead hematoxylin method of Solcia et al. (1969).

The ultrastructure of the ACTH cells resembles that of the putative prolactin cells. Electron-dense secretory granules are usually located at the cell region adjacent to the basement membrane and neurohypophysis. The granules of this cell are characterized by a separation between the granule

and its membrane (Follenius, 1968; Nagahama and Yamaoto, 1969, 1970; Abraham, 1971; Cook and Van Overbeek, 1972; Leatherland, 1970a, 1972). Secretory granules of the species investigated range from 100nm to 300nm in diameter (Knowles and Vollrath, 1966b,c; Dharmamba and Nishioka, 1968; Hopkins, 1969; Nagahama and Yamamoto, 1970; Cook and Van Overbeek, 1972; Leatherland, 1972).

In addition to prolactin and ACTH cells, thyrotropic (TSH) cells have been described in the RPD of Anguilla anguilla (Ball and Baker, 1969) and in C. auratus (Leatherland, 1972) and gonadotrops in O. nerka (Cook and Van Overbeek, 1972). TSH cells in the eels, A. anguilla and C. conger (Knowles and Vollrath, 1966b) were found to be interspersed in prolactin follicles. These cell types will be discussed greater detail with other cell types normally found in the caudal pars distalis (CPD) as described by Ball and Baker (1969).

Non-secretory agranular chromophobe cells have been described in all parts of the adenohypophysis of many teleosts: A. anguilla (Knowles and Vollrath, 1966b); M. cephalus (Abraham, 1971); G. aculeatus (Leatherland, 1970a); C. auratus (Leatherland, 1972); O. nerka (McKeown and Leatherland, 1973), but their role is unknown. These cells have also been described as neck cells, interstitial cells, or stellate cells (Leatherland, 1969, 1970a; Abraham, 1971, 1974). Knowles and Vollrath, (1966) suggested that the neck cells in A. anguilla RPD might regulate the passage of substances along intercellular channels between the prolactin cells by alterations in their shape. In the stellate form, the network of fine processes between the endocrine cells (forming a close association with the basement membrane) suggests that the cells in these forms could be involved in the movement

of hormones from endocrine cells to capillaries, (Weiss, 1965; Hopkins, 1969; Leatherland, 1970a; Leatherland et al., 1974; Batten et al., 1975).

Although all the authors cite possible roles of endocrine cell product transport, none offer the possibility of a glial or structural role.

Caudal Pars Distalis

Three cell types have been defined by tinctorial properties, granular morphology, and location in the caudal pars distalis (CPD) of most fish. Putative TSH cells have been found in O. keta and O. nerka (Nagahama and Yamamoto, 1969a) Zoarces vivparous (Otzan, 1966) Gasterosteus aculeatus (Follenius, 1968) and Carassius auratus (Nagahama and Yamamoto, 1969b; Leatherland, 1972).

The thyrotrops are probably the most variable in structure and location. Thyrotrops have the smallest secretory granules of the basophils. Their size ranges from 60nm in C. auratus (Nagahama and Yamamoto, 1969b) to 120nm in O. nerka (Nagahama and Yamamota, 1969a; 1970; Cook and Van Overbeeke, 1972). In C. auratus, Kaul and Vollrath (1974) recognized two types of TSH cells, one in the RPD and the other in the CPD. These are indistinguishable by light microscopy (Leatherland, 1972) but according to Kaul and Vollrath (1974) those in the CPD have larger granules (160nm) than those in the RPD (105nm). This distinction has not described by other observers in Carassius (Nagahama and Yamamoto, 1969b; Leatherland, 1972).

The only acidophilic cell of the CPD is the somatotrop which has been described in most of the CPD's examined. In electron micrographs, the secretory granules are noticeably larger than those in the prolactin cells.

They range in size from 200nm in O. keta and O. nerka (Nagahama and Yamamoto, 1969a; Cook and Van Overbeeke, 1972) to 460nm in O. auratus (Leatherland, 1972).

Sexually mature teleosts have cells that are usually peripherally arranged around the CPD and are generally considered to be gonadotropic in function, as judged by their cytological changes during maturation (Schreibman, 1964; Mattheij, 1970; Sage and Burns, 1971). Leatherland (1969), Pandey and Leatherland (1970), and Mackay (1971) used methallibure, an agent which inhibits the production of gonadotropins, to identify this cell in some species. Although two types of gonadotropic cells have been described in several teleosts, eg. Anguilla, Oncorhynchus, and Zoarces, (Ball and Baker, 1969; Schreibman, Leatherland, and McKeoman, 1973; Abraham, 1973; and Holmes and Ball, 1974), there is no evidence of more than one type in P. latipinna (Batten et al., 1975) or in H. fossilis (Baker et al., 1973).

In electron micrographs, gonadotropic cells of C. auratus, O. nerka, and O. keta (Nagahama and Yamamoto, 1969a, b, 1970; Leatherland, 1972; Cook and Van Overbeeke, 1972) contain electron-dense granules ranging in size from 300nm to 840nm and larger cytoplasmic vacuoles which constitute a larger percentage of the cell volume (Follenius, 1968; Nagahama and Yamamoto, 1969; Leatherland, 1970b, 1972; and Cook and Van Overbeeke, 1972).

The number of cells producing the gonadotropic hormones is as much in dispute as is the number of gonadotropic hormones produced by each cell. Recent biochemical investigations suggest that there might be only one type of gonadotropin present in fish which is capable of serving the dual role of FSH and LH (Yamayaki and Donaldson 1968a,b; Burzawa-Gerard and

Fontaine, 1972; Sundararaj and Anand, 1972). Appropriate biological activity could be achieved by one gonadotropin stimulating multiple receptor sites (Burzawa-Gerard and Fontaine, 1972).

Only one gonadotrop was defined in G. aculeatus (Follenius, 1968; Leatherland, 1970b), C. aggregata (Leatherland, 1969), O. nerka (Nagahama and Yamamoto, 1969a; McKeown and Leatherland, 1973). O. keta (Nagahama and Yamamoto, 1970) and O. latipes (Kasuaga and Takahashi, 1970). Two cell types (FSH and LH-like) were described in A. anguilla and C. conger (Knowles and Vollrath, 1966b) and in Z. viviparous (Oztan, 1966). Cook and Van Overbeeke (1972) suggested that two gonadotropic cells are also present in sexually mature O. nerka. One is distributed in both the RPD and CPD while the second, a vacuolated cell, is limited to the caudal pars distalis. Leatherland, (1972), described two gonadotropins in C. auratus which differed in size of the secretory granules.

Pars Intermedia

The pars intermedia is the most posterior of the adenohypophysial regions. Two cell types are described in most species; however, the salmonids have only one (Nagahama and Yamamoto, 1969; Cook and Van Overbeeke, 1972). In most species identification of the cell types is made by combining the staining techniques of lead hematoxylin and PAS (Sage and Bern, 1971).

Melanocyte stimulating hormone (MSH), whose source is the lead hematoxylin positive cells, is the only hormone that has been isolated and identified from cells of the pars intermedia (Olivereau, 1969a,b; Sage and Bern, 1971; Ball et al., 1972; Baker, 1972). The MSH cells of teleosts

closely resemble the MSH cells in mammals which allows easy identification (Ball and Baker, 1970, and Sage and Bern, 1971).

In addition to the lead hematoxylin cells of the PI, there is a PAS-positive cell recognizable at the light microscopic level in teleosts. Although the role of the PAS-positive cell is still unknown, Olivereau and Ball (1970) and Ball et al., (1972) suggest a possible involvement in teleostean osmoregulation.

At the ultrastructural level, two cell types are also evident in most species investigated. The PAS-positive cells contain electron-dense granules that are spherical or rod-shaped in G. aculeatus (Follenius, 1968; Leatherland, 1970b), O. latipes (Kasuaga and Takahashi, 1970), and range in size from 270nm to 400nm, in the goldfish (Leatherland, 1972) but only 145 to 215nm in Tilapia, Oncorhyncus, and Zoarces. The lead hematoxylin-positive (presumptive MSH) cell has secretory granules that range in size from 160 to 400nm in C. auratus (Leatherland, 1972).

Only two authors have described a third cell type found in the PI of teleosts. Knowles and Vollrath (1966a) and Vollrath (1966) described a cell with granule size of 150nm in the elver and adult A. anguilla.

Neurohypophysis

Scharrer (1928, 1930, 1932) first described neurosecretory cells in the hypothalamus of teleost fish. Later Palay (1945) traced the pathways of neurosecretory material throughout numerous teleost neurohypophysial systems and Bargmann and Scharrer (1951) and Bargmann (1953) substantiated a well-defined neurohypophysial system by utilizing Gomori chrome-hemato-

xylin stain on a large variety of teleosts. Since these earlier publications, the teleost neurohypophysis has attracted considerable interest due partly to availability and partly due to its unique specializations such as the penetrations of neurosecretory axons throughout the entire adenohypophysis. This is not only unique to teleosts, but is in marked contrast with the mammalian system. The use of the electron microscope lead to some significant structural and functional studies in the early 1960's notably by Lederis (1962, 1964), Knowles and Vollrath (1965a, b, c, 1966a, b) and Leatherland (1967).

The teleost neurohypophysis is comprised of unmyelinated nerve processes arising from the nucleus preopticus (NPO) or the nucleus lateralis tuberis (NLT) which lie approximately dorsal to the optic chaisma on both sides of the third ventricle (Samuelson and Fridberg, 1964; Honma and Tamura, 1965b; Knowles and Vollrath, 1966b). The greater part of the neurosecretory tract arising from the NPO contains material that is Gomori-positive and Alcian blue-positive. These fibers appear to correspond to the type A fiber described by Knowles and Vollrath (1965) which contains spherical membrane bound vesicles completely filled with electron dense contents. Measurements of the type A fibers show that two size classes can be recognized in A. anguilla. Granules measuring 160nm are found in some fishes. These were termed type A₁ fibers by Knowles and Vollrath (1965), while granules measuring 120nm in diameter characterized fiber type A₂.

In contrast to these two type A fibers, a few scattered fibers contain comparatively small vesicles of irregular form. The electron dense granules measure 60 to 80nm in diameter and do not fill the bounding membrane. Their relative electron density is less than that of the type A

vesicles. These fibers are termed type B by Knowles and Vollrath (1960, 1965a). The type B fibers do not stain with Gomori or Alcian blue stains as did the type A fibers but they do fluoresce after formaldehyde vapor treatment indicating adrenergic nerves. These fibers have been stained with a few dyes such as azocarmine, eosin, light green and with PAS-aniline blue (Stahl and Leray, 1962; Samuelson and Fridberg, 1964; Honma and Tamura, 1965b). Neurosecretory materials derived from the hypothalamic nuclei move down to axons of the preoptico-hypophysial tract in beaded form and accumulate in the neurohypophysis in large granulated structures or swellings termed "Herring bodies" (Bargmann and Scharrer, 1951; Polenov, 1960; Legait and Legait, 1957; Follenius and Porte, 1962; Knowles and Vollrath, 1965, 1966a).

In teleosts, two neurohypophysial hormones have been found in the pituitary; the octapeptides arginine vasotocin (AVT) and isotocin (IT) (Sawyer, 1966b). The arginine vasotocin is found in birds, amphibians and fishes, and differs from mammalian arginine vasopressin by one amino acid. Isotocin is only found in fishes and differs from the oxytocin found in mammals and birds by the substitution of two amino acids.

The preoptico-hypophysial tract in the more primitive A. anguilla enters the gland medially on the dorsal surface of the gland approximately in its caudal region (Dodd and Kerr, 1963; Leatherland et al., 1966; Klein, 1967). In more advanced fish like the carp, however, this relationship is altered such that the incoming stalk is enclosed by the adenohypophysis thus forming a pars nervosa core in the gland (Dodd and Kerr, 1963). The delineation between pars nervosa, infundibulum and adenohypophysis is not always easy to make. In juvenile Anguilla (Knowles and Vollrath, 1966a)

and Salmo (Klein, 1967) there is a clear division. In adults the neural component deeply penetrates in all regions of the adenohypophysial tissue (Kerr, 1949; Pickford and Atz, 1957; Legait and Legait, 1957; Leray and Stahl, 1961; Knowles and Vollrath, 1965c; Leatherland et al., 1966; Knowles, et al., 1967).

Leray and Stahl (1961) divided the pars nervosa of Mugil into two distinct regions; a rostral region and a caudal region which ramifies extensively into the pars intermedia. The rostral region contained less neurosecretory material than the caudal region. This pattern has been noted by numerous investigators in Hippocampus (Leray and Stahl, 1961; De Lage, 1955, 1958) and in Gasterosteus, Perca, Lebistes, and Anguilla (Dodd and Kerr, 1963; Follenius and Porte, 1962; Stutinsky, 1953). Electron microscopic studies by Knowles and Vollrath (1965c, 1966b) showed these nerve fibers contained irregular neurosecretory vesicles with central electron-dense granules about 70nm in diameter. These correspond to the Type B fibers of Knowles and Vollrath (1965c). The large number of nerve fibers entering the pituitary gland from the preoptico-hypophysial tract appear to enter the caudal region. These are the classic Gomori-positive (Type A) fibers which penetrate extensively into the pars intermedia (Stutinsky, 1953; Leatherland et al., 1966; Knowles and Vollrath, 1966a; Leray and Stahl, 1961; Dodd and Kerr, 1963; Sathyanesan, 1963, 1965b; Klein, 1967).

The pituicytes of the pars intermedia were well defined by Knowles and Vollrath (1965a,b). They have cytoplasm which is either electron dense and vacuolated or electron lucent and filled with fibrils. Leatherland (1972) described two types of glial cells (pituicytes) in C. auratus. Both

cells are small, with one cell having chromophobic cytoplasm and usually located in all regions of the neurohypophysis (NH). The other cell contains cytoplasmic granules which stain with PAS and are found most commonly in the dorsal NH and in the pituitary stalk. In electron micrographs, the granular type pituicytes are frequently found adjacent to blood vessels and have "active looking" Golgi bodies and little endoplasmic reticulum (ER) or mitochondria. The non-granular pituicytes have extensions of cytoplasm projecting between the neurosecretory fibers. In other species examined, C. aggregata (Leatherland, 1970c), G. aculeatus (Leatherland, 1970b), A. anguilla (Knowles and Vollrath, 1966a) only one type of pituicyte is found.

The pituicytes, which lack granules, do have numerous micro-fibrils within the cytoplasm. These cells in C. auratus also receive numerous synapses of B-type neurosecretory fibers (Leatherland, 1972). Leatherland (1972, 1970c) suggests the intimate spatial relationship of the fibrillar pituicytes with the neurosecretory fibers and basement membrane involves these cells in the transfer of neurosecretory material from the axon synapses into the basement membranes. The pituicytes containing PAS-positive material in C. auratus and C. aggregata are proposed to have a role in the engulfing of excess neurosecretory material by membrane pinocytotic specialization (Leatherland, 1972). In P. latipinna, non-secretory cells, pituicytes, had long cell processes that extended between the prolactin cells and the ACTH cells. This network also contacted processes of other pituicytes and the juxta-neurohypophysial basement membrane and its inward extensions and the bounding membrane of pericapillary spaces. The nuclei of these pituicytes were usually found among the prolactin cells and only infrequently between the ACTH cells. This organization

led Batten et al. (1975) to conclude that these stellate cells could be involved in the transport of hormones from endocrine cells to capillaries. Similar cells with indications of similar functions occur in the adenohypophysis of all vertebrates (Holmes and Ball, 1974).

The origin and destination of these fibers has recently been the subject of much investigation. The origin of the stainable or the type A fibers is most probably the nucleus preopticus (NPO) of the hypothalamus (Palay, 1945; Stutinsky, 1953; Bargmann, 1953; Stahl and Leray, 1962; Honma and Tamura, 1965b). The majority of these fibers probably terminated in the distal part of the neurohypophysis ending in close association with the pars-intermedia cells (Leatherland, 1972; Zambrano, 1972). Type A fibers have also been observed among the cells of the pars distalis (De Lage, 1958; Stahl and Leray, 1962; Sathyanesan, 1965a, 1966; Kaul and Vollrath, 1974). Type B fibers appear to originate from the nucleus lateralis tuberis (NLT) of the hypothalamus (Samuelson and Fridberg, 1964; Honma and Tamura, 1965; Knowles and Vollrath, 1966b). These fibers can be observed more frequently in the RPD and CPD than in the PI (Zambrano, 1972). The terminal endings of these fibers make synaptic like complexes with several of the pars distalis cells, pituicytes and basement membranes (Knowles and Vollrath, 1966b; Knowles et al., 1967; Vollrath, 1967; Nishioka and Bern, 1967; Zambrano, 1972).

MATERIALS AND METHODS

Channel catfish, Ictalurus punctatus weighing between 50 and 100 grams, were purchased from a commercial supplier (Ike's Fish Farm, Nashville, North Carolina), and maintained in the laboratory in a Living Stream fish tank (Frigid Units, Toledo, Ohio) at 16-18°C with constant aeration. After a period of two to three days, the fish became acclimated and were fed Purina Trout Chow daily.

Tissues for both light and electron microscopy were fixed via perfusion. For this, the fish were anesthetized with tricaine methane sulfonate (Crescent Research Chemicals, Scottsdale, Arizona). A surgical level of anesthesia was assumed when the fish was no longer able to right itself after being turned upon its back and the operculum barely moved. The fish were secured on a board, ventral surface up, and two incisions were made through the pectoral girdle with bone shears. The heart was exposed by removing the tissue covering the cardiac area. Canulation was achieved by inserting a blunt 18 gauge needle through the severed ventricle into the bulbus arteriosus where it was ligated. A fish physiological saline, (0.7% NaCl, 0.1 M phosphate buffer pH 7.4) or Cortland's balanced salt solution (Wolf and Quimby, 1969) was used to flush the circulatory system before perfusing with the fixative at the same pressure. A suitable flushing was reached when the gill lamellae blanched, usually within 2-4 minutes.

Fixation for light microscopy was accomplished by following one of the salt solutions with Bouin's fluid diluted one-to one with water. Satisfactory fixation was attained using approximately 50ml of fixative

for small fish or 100ml for larger fish. After perfusion, the cranial cavity was opened and the brain with pituitary attached was removed and placed in full strength Bouin's for one hour. The heads from a few specimens were removed, trimmed, and placed in a solution of 10% formalin and 5% nitric acid for decalcification. All tissues were stored in 70% ethanol before dehydration and embedding in paraffin. Several adult fish were perfused with half strength Bouin's followed by a suspension of india ink (6 drops/100 ml) in 3% gelatin for study of the vascular patterns of the hypophysial region. Sections were cut at 6 microns and stained by one of the following stains: Mallory's Azan, Epple's aldehyde fuchsin trichrome (Epple, 1967), periodic acid Schiff (PAS) lead hematoxylin, (Stahl, 1958) or alcian blue-PAS-Orange G (Herlant, 1960). Light micrographs were recorded on Kodak High Speed Ektachrome and Kodak Panatomic-X films.

Fixation for electron microscopy involved perfusion with half-strength Karnovsky's fixative (2% paraformaldehyde, 2.5% glutaraldehyde, buffer, pH 7.4) with 4% dextran (Bohman and Maunsbach, 1970). When the stomach and intestines were rigid, the head was removed and the brain with the pituitary still attached was removed. Tissues were placed in fixative without dextran and stored on ice for two hours at the end of which time the tissue was diced. Next, the tissue was rinsed either in three 30-minute changes or an overnight change of 0.1 M sodium cacodylate (pH 7.4). Post-fixation was in cold 1% osmium tetroxide in 0.1 M buffer (either sodium cacodylate or s-collidine) at pH 7.2-7.4 for one hour followed by three 10-minute buffer rinses allowing the last change to come to room temperature.

All tissues were dehydrated in a graded ethanol series followed by two changes of propylene oxide and embedded in Durcupan (Fluka, Columbia, South Carolina). Thick sections (0.5 - 2.0 μ m) and thin sections (65nm) were cut on glass and diamond knives, respectively, using a Reichert OMU-2 ultramicrotome. Thick sections were stained with alkaline toluidine blue for observation by light microscopy. Ultra thin sections were mounted on uncoated copper grids, double stained with uranyl acetate and lead citrate (Reynolds, 1963), and viewed with either an Hitachi HS-8 or a Philips 300 transmission electron microscope.

RESULTS

The hypophysis of the channel catfish Ictalurus punctatus lies beneath the hypothalamus and is suspended by a short infundibular stalk posterior to the optic chiasma. The gland is nestled in a small depression of the hypothalamus formed by the two lobes of the hypothalamus. Two distinct regions of the pituitary are visible to the unaided eye. The anterior region is white and corresponds to the pars distalis of mammals. The other region is larger than the anterior region and corresponds to the pars intermedia. Dorsally, the neurohypophysis, a neural extension from the hypothalamus, interdigitates all regions of the adenophysis and pars intermedia. These interdigitations with the pars intermedia are so complex that the term neuro-intermediate lobe is commonly used to describe the area. The adenohypophysis is divided into three distinct areas delineated by the morphology and tinctorial properties of the cells that populate these areas (Pickford and Atz, 1957). In sequence from anterior to posterior, these areas are the rostral pars distalis (RPD), the caudal pars distalis (CPD) and the neurointermediate lobe (NI).

Rostral Pars Distalis

The rostral pars distalis is comprised of a mass of carminophilic cells which form a cap-like area at the anterior most portion of the adenohypophysis. Interspersed among the carminophilic cells is a cell type that stains with either aniline blue - PAS, or aldehyde fuchsin. The posterior boundry of this region, forming an interface with the neurohypophysis, is formed by cells which stain with lead hematoxylin.

The carminophilic cells are the predominant cell type of this

region. These cells stain vividly with azocarmine and acid fuchsin, thus forming an obvious red mass at the anterior end of the gland. At the ultrastructural level, these cells contain secretory granules measuring 140-335nm in diameter contained within a limiting membrane and separated from that membrane by a clear space measuring 20 to 140nm (Figs 1,2). Frequently these cells are seen with their long axis lying at right angles to a capillary space or a basement membrane. In this arrangement the nucleus lies basally while the secretory granules are toward the vascular pole of the cell.

Interspersed throughout the RPD are pituicytes or glial cells which possess long extensions projecting between many of the carminophilic cells. The cytoplasm and nuclei of these pituicytes are relatively electron dense.

The second cell type of the RPD is found randomly dispersed throughout the carminophilic cells (Fig. 1). They appear singly or in small clusters and can be identified by their affinity for aniline blue (AB), PAS, and aldehyde fuchsin. At the ultrastructural level, the cell can be identified by small secretory granules which measure 90 to 285 nm in diameter and exhibit highly variable electron density.

The third cell type lies along the rostral pars distalis-caudal pars distalis interface; however, occasional cells of the third type are also found deeper within each area (Fig. 3). These cells stain only with lead hematoxylin. The secretory granules are large and measure 145-335nm in diameter for the spherical granules while the rod and dumb-bell shaped granules measure to 800nm in length. These cells separate the neurohypophysis

from the carminophilic cells of the rostral pars distalis and can also be found occasionally within the neurohypophysis.

The cap-like RPD is separated from the caudal pars distalis by a pronounced basement membrane that runs dorso-ventrally through the entire organ.

Caudal Pars Distalis

There are four distinct cell types in the caudal pars distalis (CPD), in addition to the lead hematoxylin cells which border the RPD-CPD interface. These cells form a distinct band running from the antero -ventral surface of the adenohypophysis to the postero -dorsal surface. Within this region there is one type of acidophil and three types of basophils. The acidophils stain vividly with Orange-G and are scattered throughout the caudal area. At the EM level, the Orange-G positive cell has granules ranging in size from 150-285nm (Fig.4). The granules are electron dense and have shapes ranging from spherical to ellipsoid with an occasional dumb-bell shape observed. The three basophils are not distinguishable as individual cell types at the light microscopic level. Collectively they stain with PAS, aldehyde fuchsin, aniline blue or alcian blue in common trichrome or tetrachrome techniques.

At the ultrastructural level, the three basophils have secretory granules which are less electron dense than the acidophils or the lead hematoxylin positive cells. One basophil cell type of this region has secretory granules ranging in size from 100 to 190nm (Fig.4). The second basophilic cell type has granules which are 100 to 280nm in diameter and vary in electron density from dark to faint and indistinct (Fig.5). The third baso-

philic cell has granules ranging in size from 90 to 190nm. These granules are the same size as type one but also appear to be slightly more electron dense than those of the first type of basophil.

In addition to the endocrine cells of the CPD there are also two distinguishable chromophobes in the pars distalis (Figs. 11,12). These are non-granular, have deeply involuted nuclei, and either lightly or darkly staining nuclei and cytoplasm. These cells lie among the putative secretory cells sending long cytoplasmic projections deeply between other endocrine cells.

Neurointermediate Lobe

The neurointermediate lobe has two cell types distinguishable at the light microscopic level. The first cell type has large granules and stains with PAS only. The other cell type is smaller than the first, is more numerous, and stains with lead hematoxylin. Both cell types are evenly dispersed throughout the region.

At the EM level three cell types are distinguishable, the first having large electron dense granules ranging in size from 190 to 380nm in size (Fig. 8). The second cell type has granules ranging in size from 100 to 190nm and vary in electron density from light to dark with a distinct limiting membrane surrounding each granule (Fig.9). The third cell type found in this area has granules ranging in size from 100 to 190nm and relatively electron lucent with larger inclusions ranging in size from 235 to 475nm (Fig.10). These were the most sparse of the three cell types.

Nerve Supply

Nerve fibers descending from the hypothalamus innervate all regions of the pituitary. Two types of neurosecretory fibers come into

either direct or indirect contact with adenohypophysial cells. One type of fiber Type A stains with aldehyde fuchsin or alcian blue after acid permanganate oxidation. The other, Type B, fiber does not stain with the "classic" neurosecretory stains. The former type of fiber generally passes through the adenohypophysis into the intermediate or posterior lobe where the fibers branch. In the neurointermediate lobe these fibers traverse the basement membrane which separates the neurohypophysial core from the anterior lobe.

At the EM level, the first type of fibers contain typical neurosecretory granules of 150 to 200nm in diameter and usually terminate in the distal part of the neurointermediate lobe (Figs.8,9,10). In some instances they can be seen in intimate contact with the endocrine cells having electron lucent granules, type 2 (Fig.9). These same fibers were not seen innervating the glandular cells of the adenohypophysis. The second fiber type contains small electron lucent vesicles 90nm in diameter and a few electron dense granules 90 to 150nm in diameter (Figs. 1 through 7). These fibers traverse the basement membrane of the neurohypophysial core and spread throughout the adenohypophysis and neurointermediate lobe where they come into intimate contact with glandular cells. In some cases these fibers terminate on the basement membrane near glandular cells (Figs.2,3,4). The membrane specializations seen at points of contact are termed synaptic because they show synaptic vesicles and resemble the synaptic complexes found in the central nervous system (Figs.4,5,6).

Structures resembling synaptic complexes including membrane specializations and synaptic vesicles were observed between some type A fibers and the basement membrane in the CPD and to the type 1 cells (presumptive

MSH cells) of the neurointermediate lobe (Fig.9). Type B synaptic complexes were observed between the nerve fibers and the basement membrane in both the RPD and the CPD. Type B fibers also formed complexes with type 1 cells (presumptive prolactin cells) of the RPD (Fig.2), type 2 cells (presumptive TSH cells) (Fig.5), with types 1 (presumptive MSH cells) (Fig.9), 2 (Fig.8), and 3 (Fig.10) of the neurointermediate lobe, and with the type 3 (presumptive gonadotropic cell) basophil of the CPD (Fig.4). There was also a synaptic complex formed with the lightly staining chromophobe.

The neurosecretory axonal fibers run parallel to one another and end in swollen terminal buds on or near the basement membrane. The axons contain microtubules, mitochondria, and small numbers of membrane bound electron dense granules, whereas the swollen terminal buds contain large numbers of granules together with mitochondria and small clear synaptic-like vesicles with diameters ranging in size between 50 and 90nm.

DISCUSSION

Ten morphologically distinct secretory cells can be identified in the Ictalurus punctatus pituitary which are comparable in secretory granule morphology and staining reactions with cells of other teleost pituitaries. The cells are grouped into 3 regions, a fact that greatly aids in the identification of cells. Because the staining of the cells in these three regions is similar to that of other teleosts, it is assumed in the present study that the acidophilic cells of the rostral pars distalis produce prolactin, the lead hematoxylin cells of the border region of the RPD and CPD are adrenocorticotrops, the basophilic cells of the rostral pars distalis are thyrotrops, the basophils of the CPD are gonadotrops, and the acidophils of the CPD are somatotrops. One cell type of the pars intermedia is a melanotrop and the other cell type has an unknown secretory material. Stellate cells lacking secretory granules were observed between the endocrine cells in all regions.

Cell location and secretory granule size were major features used to identify the cells types. It must be noted that secretory granule size alone is not sufficient for conclusive identification due to significant variances in physiological states of the cells, planes of section and fixation processes. However, granule size in addition to staining reactions yield evidence to make presumptive identifications. More certain identifications can be made by immunohistochemistry as developed by Emmert (1969).

Rostral Pars Distalis

There is no difficulty in identifying the presumptive prolactin cells of the pars distalis. These cells react intensely with azocarmine,

acid fuchsin, and erythrosin. The location of these cells and their staining reaction to acid dyes results in a unique red mass of cells at the anterior end of the gland. Their ultrastructure corresponds closely to that described by other authors, however, the implied uniformity of the cell's size and shape was not found in this study. Schreibman et al. (1973) noted that such an implied morphological uniformity may be misleading due to the fact that "although presumably similar in function, cells differ in their dimensions, shape of nuclei, degree of granulation, and manner and time in which they respond to physiological challenge." The granule size (140-335nm) found in this study has a larger range than that found in other studies, with the possible exception of those found in M. cephalus (Abraham, 1971). Changes in prolactin granule size with salinity changes have been described in several teleosts including P. latipinna in which Hopkins (1969) interpreted his results as evidence that granule release was correlated to osmoregulation. In fish adapted to freshwater from saltwater, the granules were smaller and had a translucent ring between the dense core and the limiting membrane suggesting granule release before attaining mature storage size. Fish fully adapted to freshwater media in contrast, have consistently larger granules in freshwater than in high salinities (Holmes and Ball, 1974; Nagahama et al., 1973; Leatherland et al. 1974; and Abraham, 1971). The role of prolactin in osmoregulation was first described by Pickford and Atz (1957) after studying the effects of hypophysectomy in fish in freshwater and saltwater.

The identification of presumptive ACTH cells to the lead hematoxylin cells of the RPD also presents few difficulties. Most of the

catfish ACTH cells have the same distribution typical of other teleosts, namely along the RPD/CPD interface. The secretory granules are similar in size (145-335nm) to those other authors have reported; however, there was a marked absence of the wide separation of the secretory granule core from its limiting membrane as described previously (Dharmamba and Nishioka, 1968; Leatherland et al, 1974; Nagahama and Yamamoto, 1969a). Schreibman et al, (1971) and Cook and Van Overbeeke, (1970) reported a PAS-positive basement membrane separating the ACTH cells from the neurohypophysis. In this study, a basement membrane is seen in this region, however it was not possible to determine if it was continuous, thus forming a complete separation between the ACTH cells and the neurohypophysis.

To date, there remains some controversy as to which cells are involved in TSH formation. The basophilic cells of the RPD and those of the CPD were suggested to be TSH producing cells by Knowles and Vollrath, (1974). The results of the present study suggest that two groups of cells should be considered as presumptive TSH cells. The occurrence of alcian blue staining in both RPD and CPD and one ultrastructurally distinct cell type common to both regions led to the tentative identification of the TSH cell. TSH cells are also described in the RPD of *Anguilla* (Knowles and Vollrath, 1966) and the salmonids (Nagahama and Yamamoto, 1969a, 1970). Kaul and Vollrath (1974) reported that the granules of the TSH cells in the CPD of *Carassius auratus* have larger granules (mean diameter $160 \pm 15 \text{nm}$) than those of the RPD ($105 \pm 12 \text{nm}$). This distinction was not noticed by other investigators. In this study the presumptive TSH cells of the CPD have granules ranging in size from 100-190nm while the presumptive TSH cell

granules of the RPD have granules ranging in size from 90 to 285nm. In many species thyrotrops have the smallest granules of the basophils, however granule size has been shown to depend on the functional state of the cell (Hemme, 1972).

Caudal Pars Distalis

Since in teleost fish, there is just one acidophil present in the CPD there can be little doubt that this cell is the presumptive source of growth hormone. The STH cells can be easily distinguished from prolactin cells by their position and their tinctorial affinity for Orange G in the Heidenhain azan method. In electron micrographs, somatotropic granules are generally markedly larger than the granules of prolactin cells. However in this study the granules of the presumptive somatotrops were smaller than those of the presumptive prolactin cells. Also, the halo surrounding the prolactin cell granules was absent from the somatotrops.

The number of gonadotropic hormones and the number of cells producing them remains a mystery. Histochemical investigations conducted by Yamazaki and Donaldson (1968a,b), Burzawa-Gerard and Fontaine (1972) and Sundararaj and Anand (1972) suggest that there may be only one type of gonadotropin produced which has the capability of acting both as FSH and LH. These reports are based upon experiments involving sexually mature specimens of salmon, carp, catfish, goldfish. In O. kisutch, however, two different gonadotrops have been identified. At the ultra-structural level, one gonadotrop was identified in G. aculeatus (Follenius, 1968; Leatherland, 1970b) C. aggregata (Leatherland, 1969), O. nerka

(Nagahama and Yamamoto, 1969a), and O. keta (Nagahama and Yamamoto, 1970). Two gonadotrops were described in Anguilla and Conger (Knowles and Vollrath, 1966b) and in C. auratus (Leatherland, 1972). Variances in cell and granule size in addition to dilated rough endoplasmic reticulum, sometimes considered a criterion for cell activity, could be attributed to degrees of maturation and thus makes it difficult to determine the number of functional gonadotrophs. Since the present study was conducted on immature fish, correlations between I. punctata and other fish is therefore difficult.

Two cell types remain to be identified in the CPD in this study. In both of these cells, the granules are approximately the same diameter (100-190nm), but appear different in granule density. It is possible that this is due to the young age and therefore early state of development of the reproductive organs. Therefore these cells could represent two distinct cell types or one cell type at different stages of development.

Non-secretory cells have been described in both regions of the adenohypophysis of many teleosts, sometimes as stellate cells, neck cells, interstitial cells, or chromophobes. (Leatherland, 1969, 1970a; Abraham, 1971, 1974). Weiss (1965) and Hopkins (1969) conjectured that in P. latipinna, the network of projections from the chromophobe extending between the endocrine cells and the basement membrane and pericapillary spaces might be involved in the transport of hormones from the intrinsic endocrine cells to capillaries. The long projections interdigitating the endocrine cells parallels the patterns described by Weiss (1965) and Hopkins (1969). The projections are particularly noticable leaving the cells laying adjacent to a basement membrane surrounding the vascular spaces. These projections can often be traced deeply among the cells of either region. Another

possible function of the deep projections from chromophores is the isolation of endocrine cells into functional groups. No function can be designated to the two different types of chromophores found in this study. They both possess nuclei with little heterochromatin and cytoplasm devoid of any secretory granules. The major difference is in the apparent electron density of the cytoplasm. The cytoplasm of one cell appears much darker than the others. This might be due to differences in the rate of uptake of the stains or physiological state of the cell. However it is interesting to note that it was not at all uncommon to find the two types of cells lying next to each other.

Pars Intermedia

As in other teleosts, the posterior-most region of the adeno-hypophysis is the pars intermedia. Sage and Bern (1971) using a combination stain of lead hematoxylin and PAS identified two cell types; one cell was reactive to the lead hematoxylin while the other was PAS positive. Evidence from the studies on A. anguilla (Olivereau, 1969a, b.; Ball et al, 1972; and Baker 1972) has indicated that the lead hematoxylin positive cell is the source of MSH. The role of the PAS-positive cell has yet to be clearly defined, but Olivereau and Ball (1970) have suggested a possible role in osmoregulation.

At the EM level two cell types have been identified in G. aculeatus (Follenius, 1968; Leatherland, 1970b), O. latipes (Kasuga and Takahashi, 1970), O. keta (Nagahama and Yamamoto, 1970), O. nerka, (McKeown and Leatherland, 1973) and C. auratus (Leatherland, 1972). The PAS-positive cells of G. aculeatus and O. latipes are filled with electron dense

spherical or rod shaped granules. The presumptive MSH cells, however, contain granules ranging from very dense to faintly electron dense. A third cell type was described by Knowles and Vollrath (1966a) and Vollrath (1966), but the authors suggested that these cells were undergoing degeneration.

In this study of Ictalurus, the granule size of the presumptive lead hematoxylin cells (100-190nm) is smaller than those described in C. auratus (Leatherland, 1972); Z. viviparous (Oztan, 1966); and O. keta (Nagahama and Yamamoto, 1970). The secretory granules ranged greatly in density with several granules demonstrating a halo between the granule and its membrane. These cells were more numerous than either of the other two types.

The presumptive PAS-positive cells have approximately the same size granules as those found in C. auratus. However the granules in I. punctatus are larger than those described in Tilapia sp. (Schreibman, Leatherland, and McKeown, 1973); Z. viviparous (Oztan, 1966); or O. keta, (Nagahama and Yamamoto, 1970).

The third type of granulated cell in this study did not show signs of degeneration, but did exhibit secretory granules that reacted only faintly with EM staining techniques employed. These cells were not numerous and did appear widely scattered throughout the region.

The variances of granule size in the two main secretory cell types may possibly be due to their roles in osmoregulation as suggested by Olivereau and Ball (1970) and Ball et al. (1972), or in pigment control (Olivereau, 1971; Baker, 1972; Thornton and Howe, 1974).

The neurohypophysis of Ictalurus punctatus closely resembles that of Poecilia latipinna, Anguilla anguilla, Tilapia mossambica, Gill-ichthys sp., and Gasterosteus sp. Nerve fibers entering the adenohypophysis from the infundibulum arise from the nucleus preopticus (NPO) as described in the eel by Knowles and Vollrath (1966b) and the nucleus lateralis tuberis (NLT) (Knowles and Vollrath, 1966a; Sathyaneson, 1969). As indicated in previous reviews (Perks, 1969; Dodd, Follett, and Sharp, 1971), the fibers arising from the NPO stain with aldehyde fuchsin, or alcian blue after permanganate oxidation and seem to correspond to the type A fibers described first in Anguilla (Knowles and Vollrath, 1966a,b). These fibers are peptidergic and the presumable source for the octapeptides arginine vasotocin (AVT) and isotocin (IT) (Zambrano 1970a,b; Bern, Zambrano, and Nishioka, 1971; Zambrano, Nishioka, and Bern, 1972). Fibers arising from the NLT react positively to the Falck-Hillarp technique (Falck, 1962) for identifying catecholamines and appear to correspond to the type B fibers as seen in Anguilla (Knowles and Vollrath, 1966a,b). These fibers are adrenergic as confirmed by the administration of 6-hydroxydopamine leading to the depletion of monoamines.

Since no investigation of the hypothalamus to determine the origins of the nerves (supplying) the pituitary were undertaken in the present study, it is impossible to comment with certainty on the origin of the nerve fibers in Ictalurus. However, some evidence exists which would substantiate the findings in other fish examined to date. There were two cell types found, one stained vividly with aldehyde fuchsin and the other stained with alcian blue and fluoresced with pseudo-isocyanin. These cells probably give rise to type A and type B fibers respectively.

Knowles and Vollrath presented evidence for two groups of A fibers, one with typical elementary neurosecretory granules (ENG) ranging from 160-180nm in diameter, and others with granules 120nm in diameter. The measurements taken on the Ictalurus type A granules did not allow for such a distinction since granule size ranged from 150 to 200nm.

The granule size (90nm) of the presumptive type B fibers in Ictalurus corresponds to those described in Anguilla and Poecilia. The secretory granules found within these fibers are large granulated vesicles (LGV) with a clear space between the granule and the bounding membrane. Similar LGV have been described in Gillichthys (Zambrano, 1970a) and Anguilla (Knowles and Vollrath, 1966b).

The nature of the innervation of the nerve cells is of interest due to its contrast with mammalian systems. In Ictalurus, as in other teleosts, the nerve fibers arising from the hypothalamus terminate in various ways on or near the endocrine cells within the pituitary. Type A endings predominate in the pars intermedia, while type B endings predominate in the pars distalis.

In reviews by Ball and Baker (1969) Bern, Zambrano, and Nishioka (1971) and Leatherland (1970b,c), the range of neuroglandular junctions is from direct cell contact, sometimes as a synaptic junction, to direct contact to the double basement membrane also in a synaptic manner. In many fish observed to date (Ball and Baker, 1969,; Leatherland, 1969, 1970c; Bern, Zambrano, and Nishioka, 1971), few type A fibers have penetrated the basement membrane separating the neurohypophysis from the pars distalis.

Those fibers have usually ended on perivascular spaces adjacent to the endocrine cells. No such endings were seen in the Ictalurus RPD and only a relative few type A nerve fibers were seen forming complexes to the basement membrane in the CPD.

Identification of type B fibers and synaptic junctions in other fish (Ball and Baker, 1969; Leatherland, 1969, 1970; Bern, Zambrano, and Nishioka, 1971) yields little consistency to identifying a pattern of junctions. For example in Gillichthys, the type B axons cross the basement membrane and make synaptic complexes with the prolactin and the ACTH cells. In Tilapia, on the other hand, axons do not terminate on prolactin or ACTH cells, but on the basement membrane or on a projection of a glial cell near the endocrine cell. In other species, direct contact has been noted on all adenohypophysial cells. As noted earlier, type B nerve fiber synaptic complexes were observed with the basement membrane in both the RPD and CPD. No such junctions were observed in the neurointermediate lobe. Complexes were also observed with type 1 cells (presumptive prolactin cells) and type 2 cells (presumptive TSH cells) of the RPD, type 3 cells (presumptive gonadotrophs) of the CPD and to all cell types of the NI lobe. In addition a synaptic junction was observed with a light staining chromophobe of the NI lobe.

Dual innervation was reported in both Tilapia and Gillichthys (Bern, Zambrano, Nishioka, 1971) on MSH cells of the PI. Knowles and Vollrath (1966a) also reported dual innervation in the eel, however synapses were on a basement membrane separating endocrine cells. Single axon innervations showing synaptic complexes by type A fibers on PAS positive

cells were reported in Gasterosteus while non-synaptoid endings on the lead hematoxylin positive cells were noted (Leatherland, 1970b). In Ictalurus, synaptic complexes can be seen between type A and B fibers and the PAS positive cells and between type B and lead hematoxylin positive cell. It can be assumed, as in other teleosts, that the endocrine cells of the NI lobe are under direct control by the hypothalamus.

Several authors (Knowles and Vollrath, 1966b; Leatherland 1969, 1970a; Abraham, 1971) have described cells which lie throughout the adenohypophysis, often lying adjacent to endocrine cells, while sending cellular processes deeply between other endocrine cells. It has been proposed that these cells are involved in intercellular transport of endocrine cell products to capillaries or material from neurosecretory terminations to target cells. Although variously named as neck cells, chromophobes or interstitial cells these cells all probably belong to a stellate cell system not fully understood at this time. In Ictalurus, numerous chromophobic cells with long processes were seen in the adenohypophysis often with the processes touching cells distantly removed from the cell body of the stellate cell. Synaptic complexes were type B fibers were occasionally observed, but no function other than one of a supporting matrix could be assigned to either the light or dark chromophobe.

Endocrine cell types of the channel catfish, Ictalurus punctatus have been described according to granular morphology and location within the gland. In addition, innervation of the pars distalis and neurointermediate lobes was described. The results of this study have shown the catfish hypophysis is similar to the model teleost fish A. anguilla and P. latipina.

Figure 1. Rostral Pars Distalis. Presumptive prolactin cell (PROL) lying adjacent to the basement membrane (BM). This cell demonstrates one orientation of the PROL cells with a polar arrangement of secretory granules. The presumptive throtropic cell (TSH) is also found in the RPD. A type B nerve fiber contacts the basement membrane adjacent to the PROL cell. 4200X

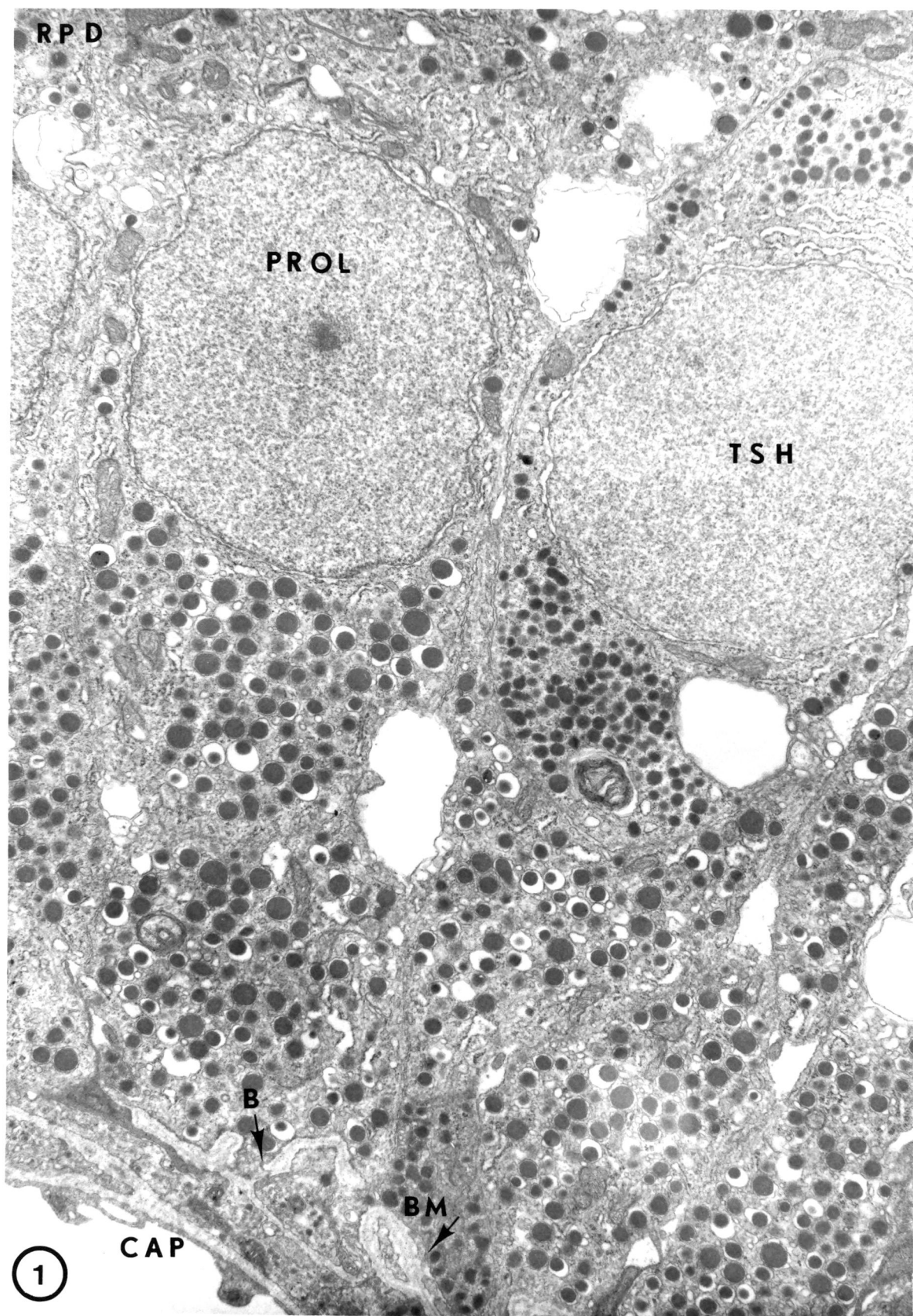


Figure 2. Rostral Pars Distalis. The second orientation of the prolactin cells (PROL) is ovoid with secretory granules distributed throughout the cytoplasm in lieu of the polar arrangement of Fig. 1. Note the presence of the basement membrane (BM) and Type B nerve fibers (B) with secretory vesicles. 4200X.

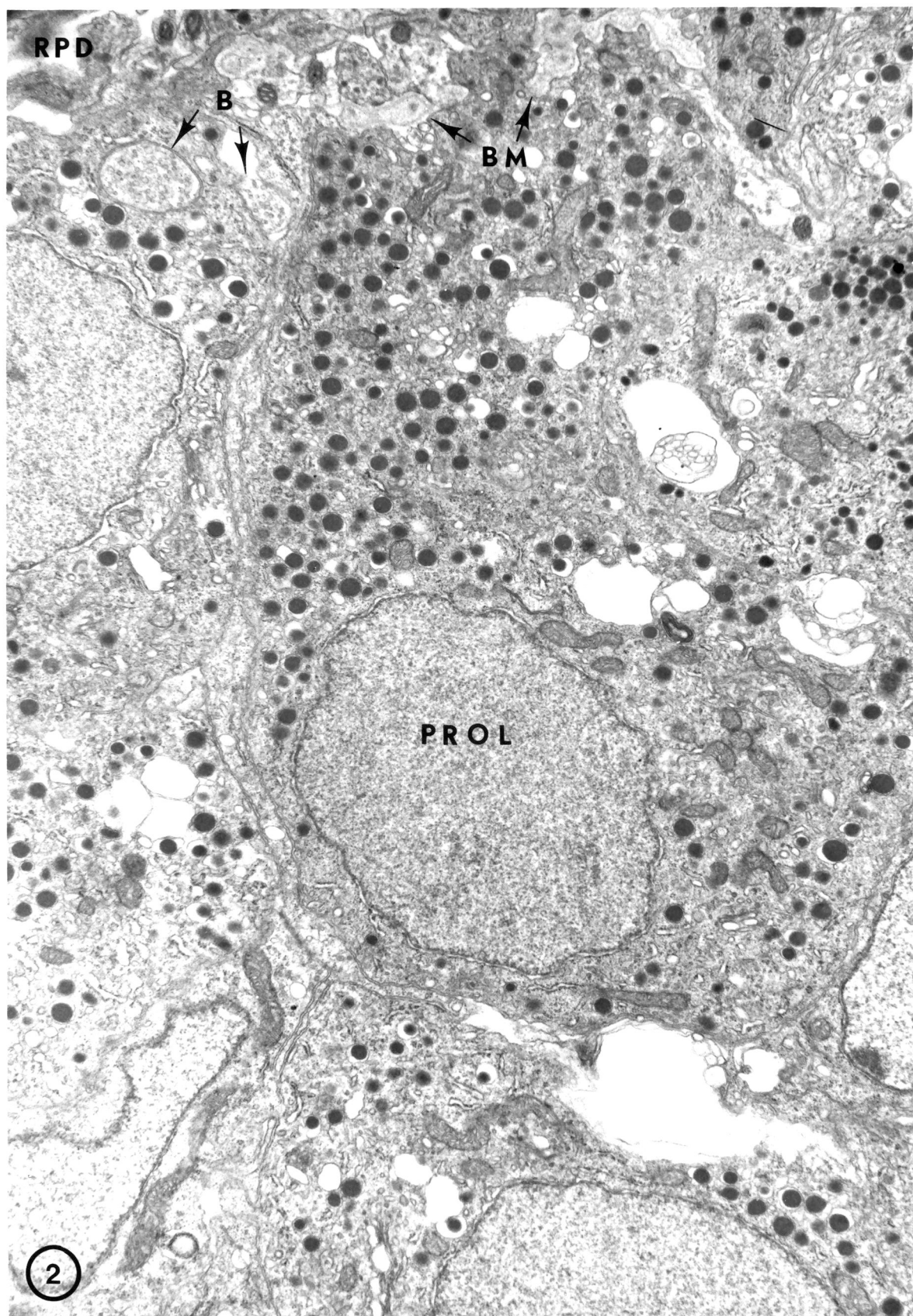


Figure 3. Rostral Pars Distalis. The presumptive adrenocorticotrophic cell (ACTH) which lies along the RPD - caudal pars distalis (CPD) interface and along the adenohypophysis - neurohypophysis interface. Note the projections of the basement membrane (BM) into the inter-cellular spaces of endocrine cells. Type B (B) nerve fibers can be seen in contact with the ACTH cells. 4200X

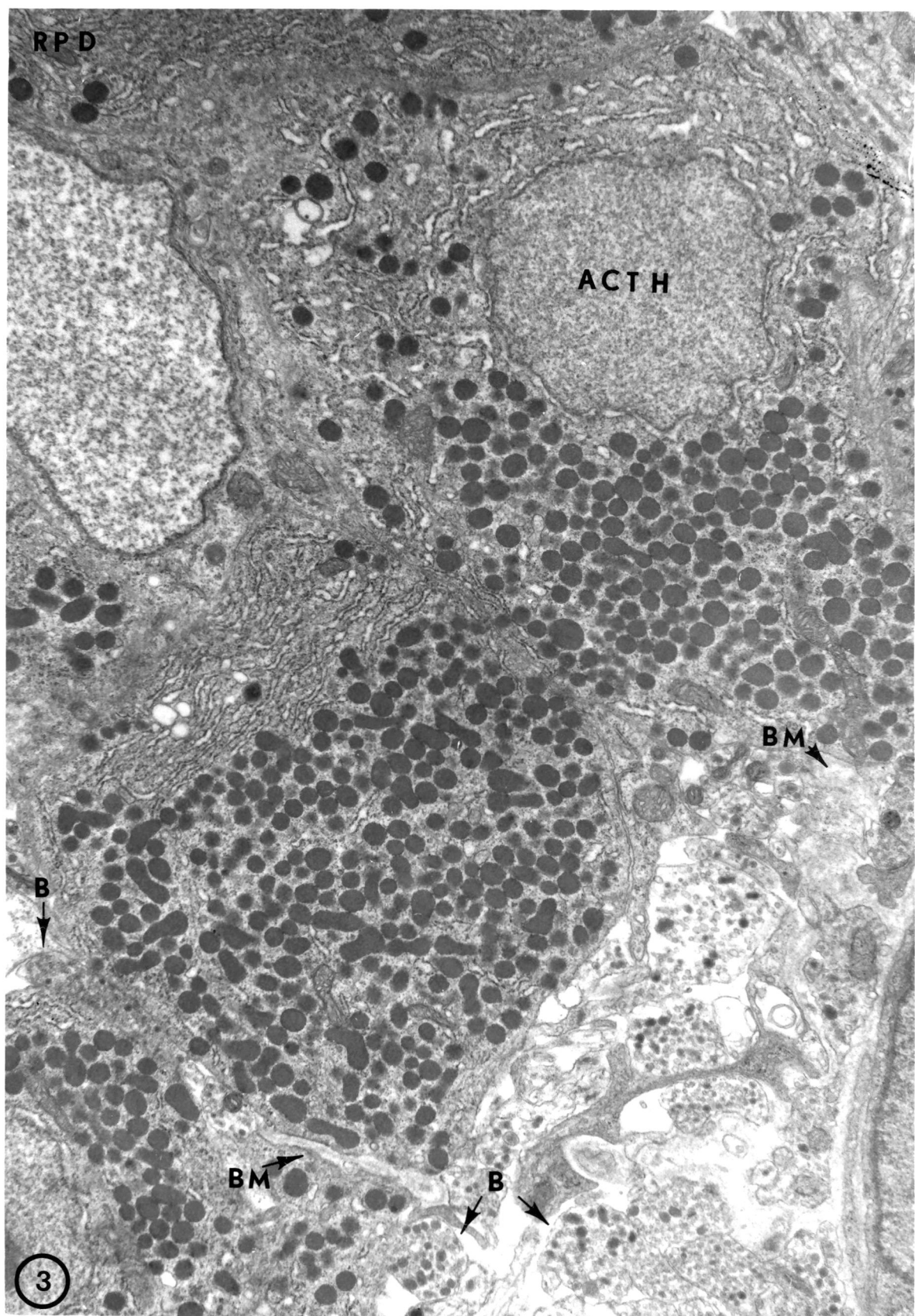


Figure 4. Caudal Pars Distalis (CPD). Presumptive gonadotropic (G) and somatotrophic cells (STH) are randomly scattered throughout the CPD. Numerous Type B fibers can be seen with neurosecretory vesicles in direct contact with these cells. 4200X.

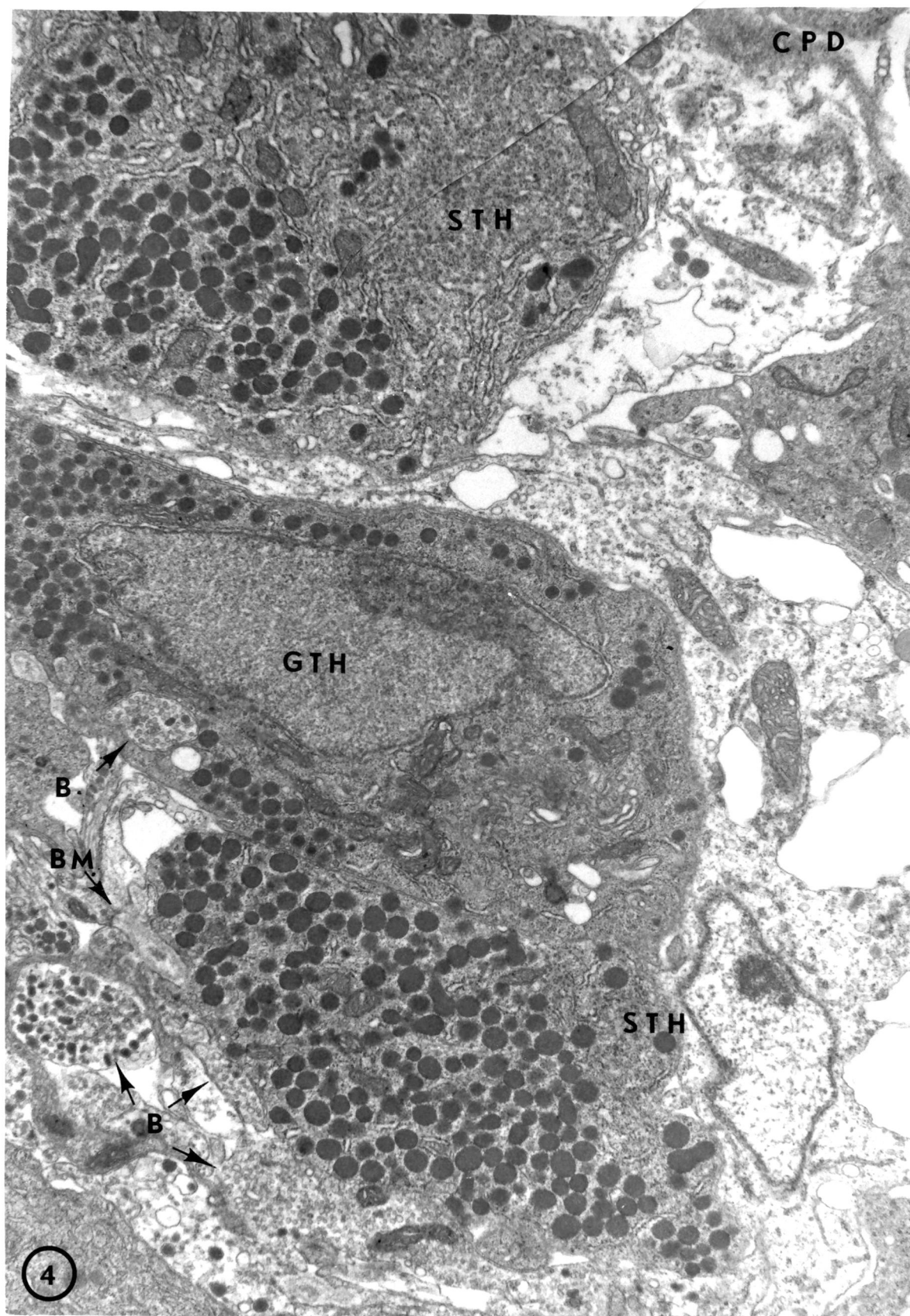


Figure 5. Caudal Pars Distalis (CPD). A presumptive TSH cell like those seen in the RPD. This is the only cell that is distributed throughout both regions. Type B fibers are also seen making synaptic contact with the TSH cell. 4200X.

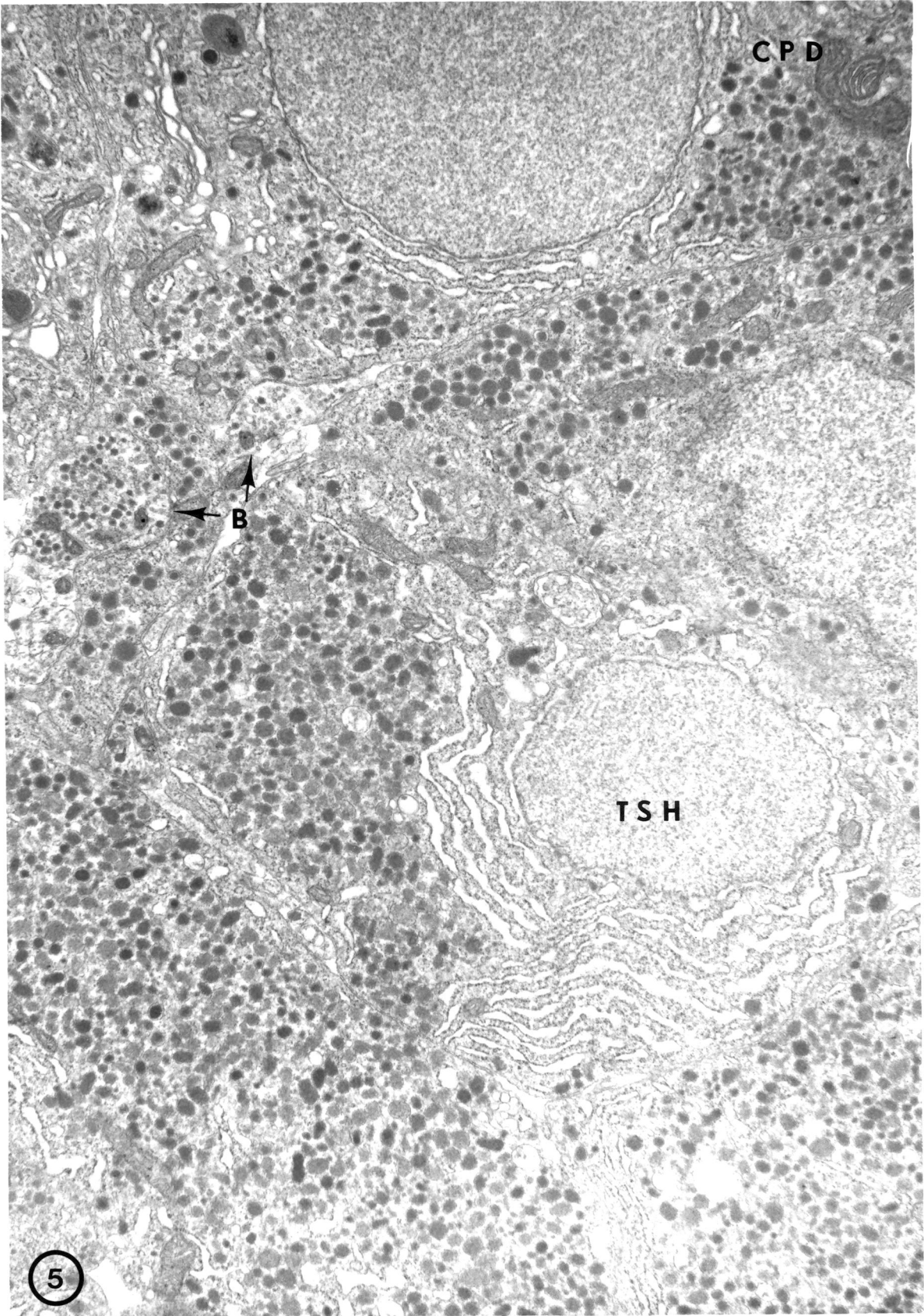


Figure 6. Caudal Pars Distalis (CPD). Presumptive TSH and gonadotropic (G) cells. The gonadotropic cells were found scarcely distributed throughout the CPD. As in the other micrographs, type B fibers contact the endocrine cell. 4200X.

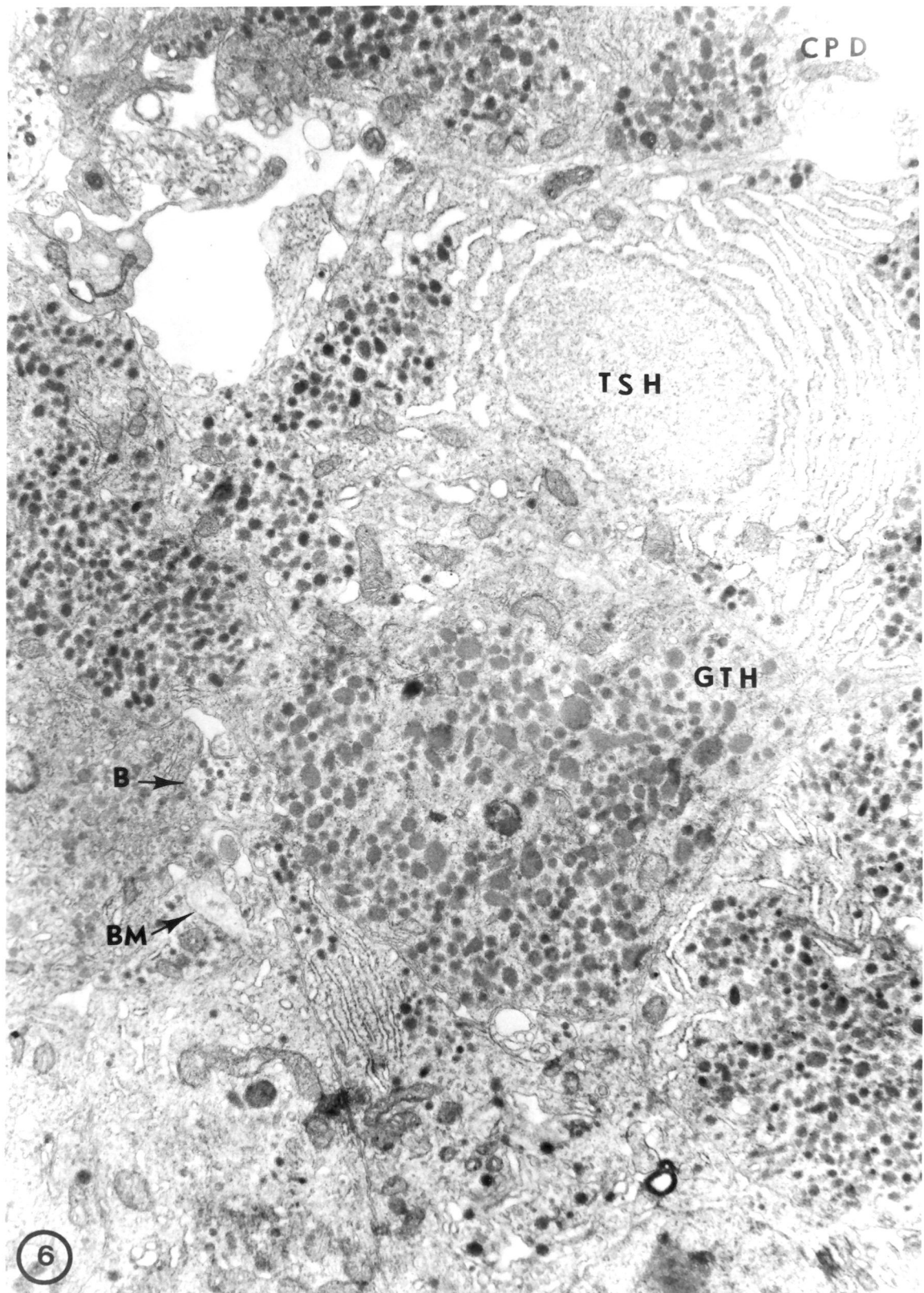


Figure 7. Caudal Pars Distalis (CPD). A typical arrangement of a capillary (CAP) surrounded by a basement membrane (BM) and cords of nerve fibers, in this case Type B fibers. 4200X.

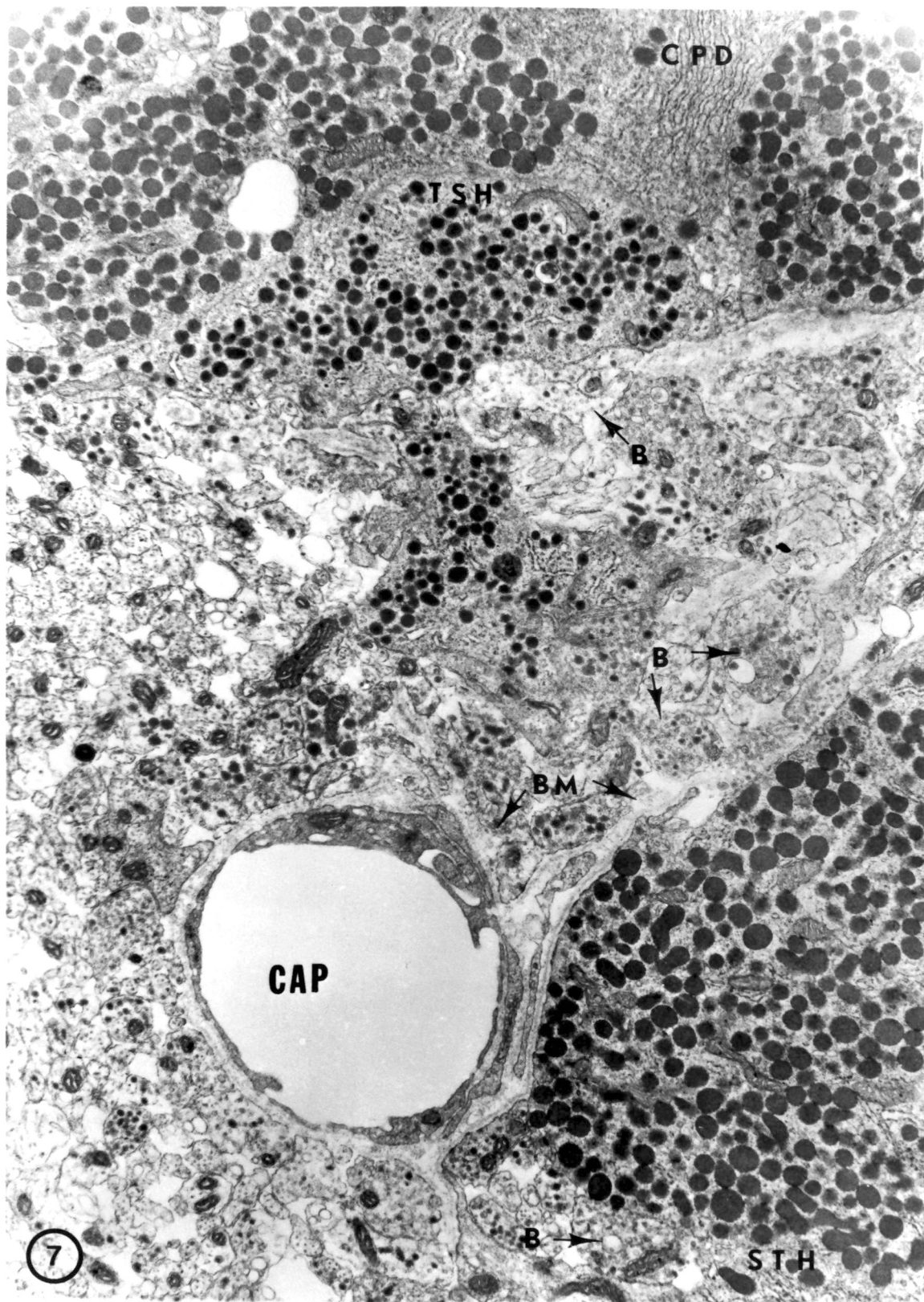


Figure 8. Neurointermediate lobe (NI). The periodic acid schiff (PAS) positive cell is one of the two predominate cell types of the NI lobe. Unlike the adenohypophysis, both type A and type B nerve fibers are found within this region. Both nerve fibers in this micrograph demonstrate vesicles plus membrane specializations at the synapse. 4200X

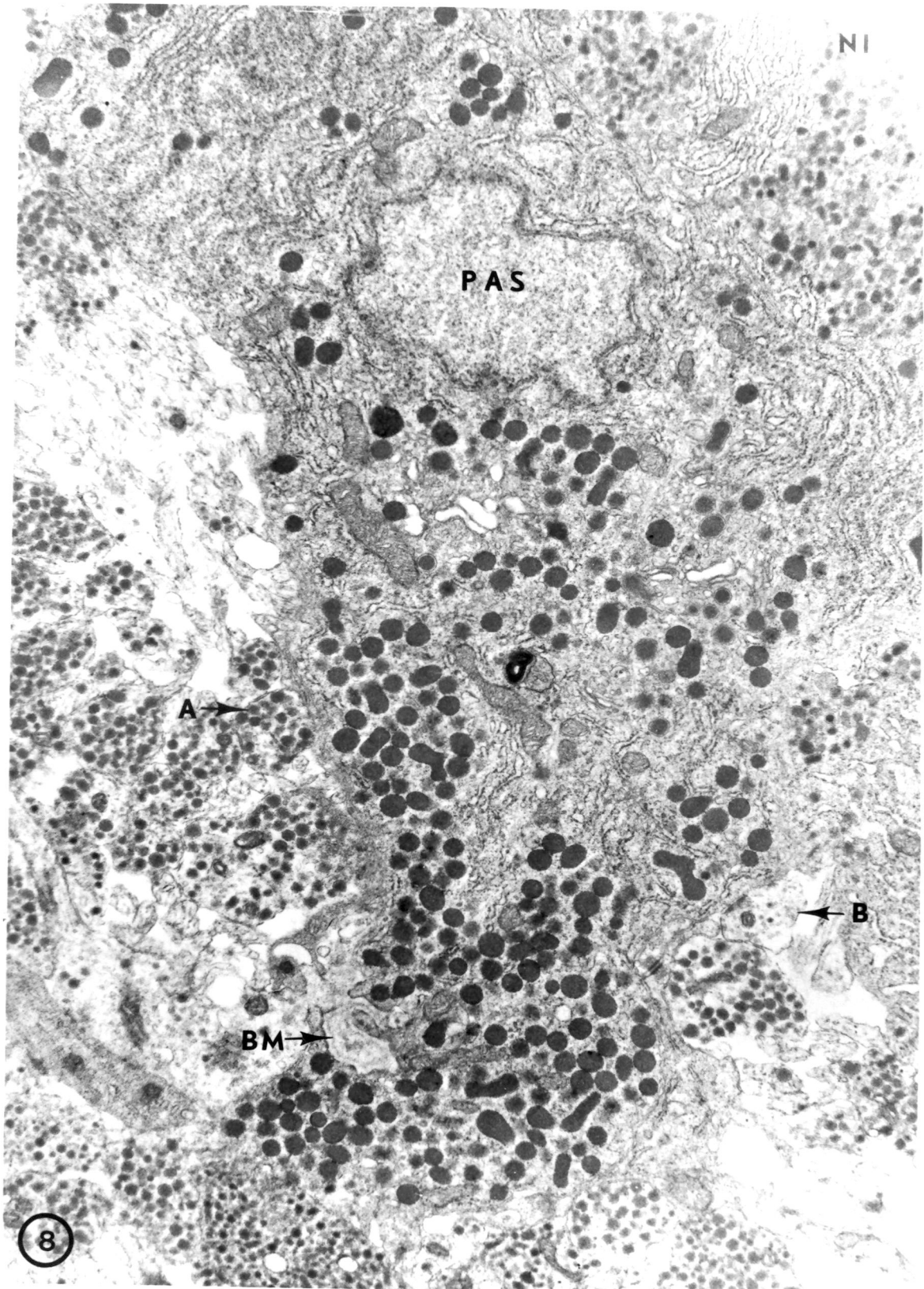


Figure 9. Neurointermediate lobe (NI). The lead hematoxylin (PbH) positive cell is the second predominate cell of the NI lobe. Both Type A and Type B make contact to this cell showing the possibility of dual control. 4200X.

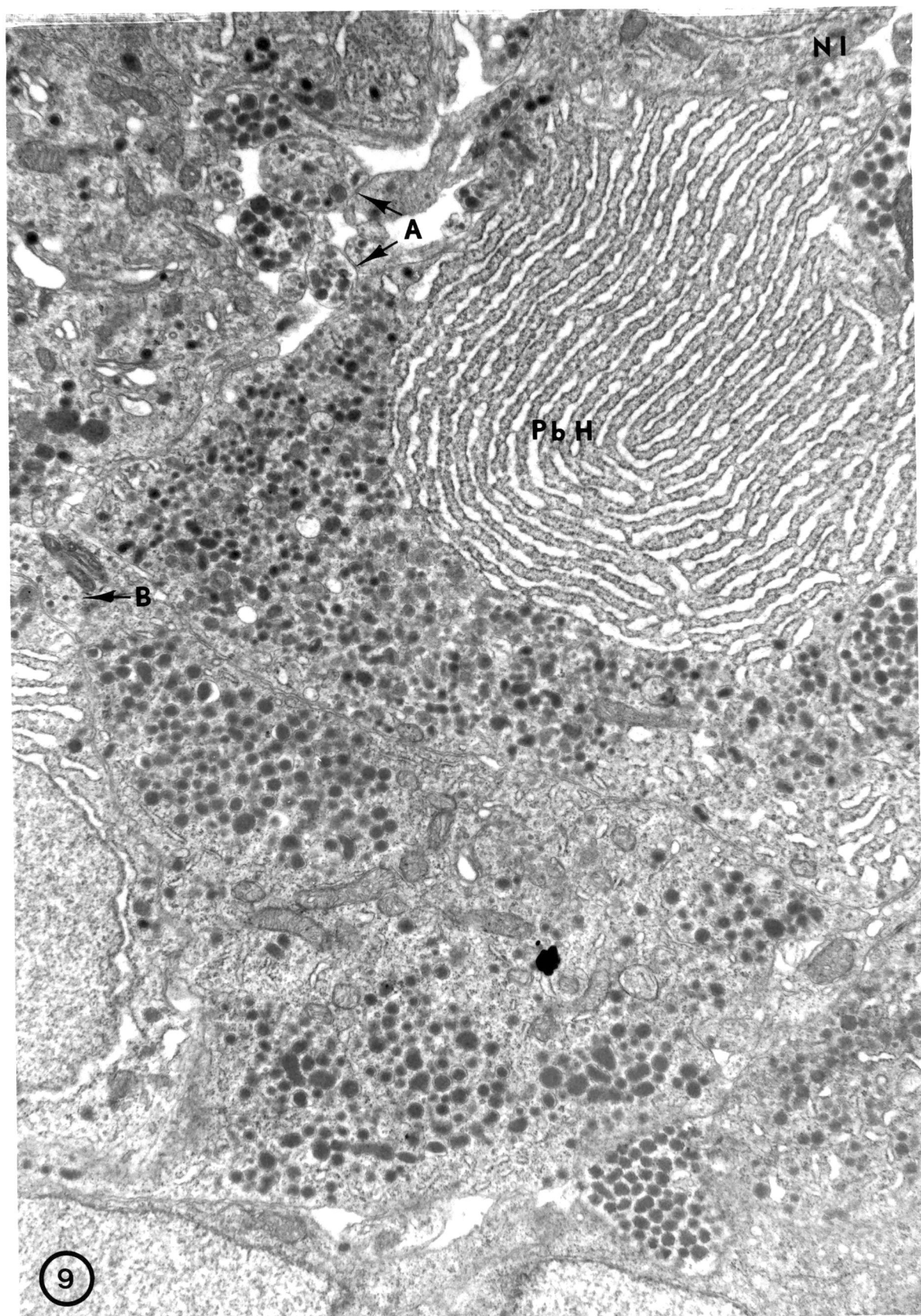


Figure 10. Neurointermediate lobe (NI). The third cell type is infrequently observed, but is found throughout the NI lobe. As with the other two cell types, Type A and Type B nerve fibers contact this cell. 4200X.

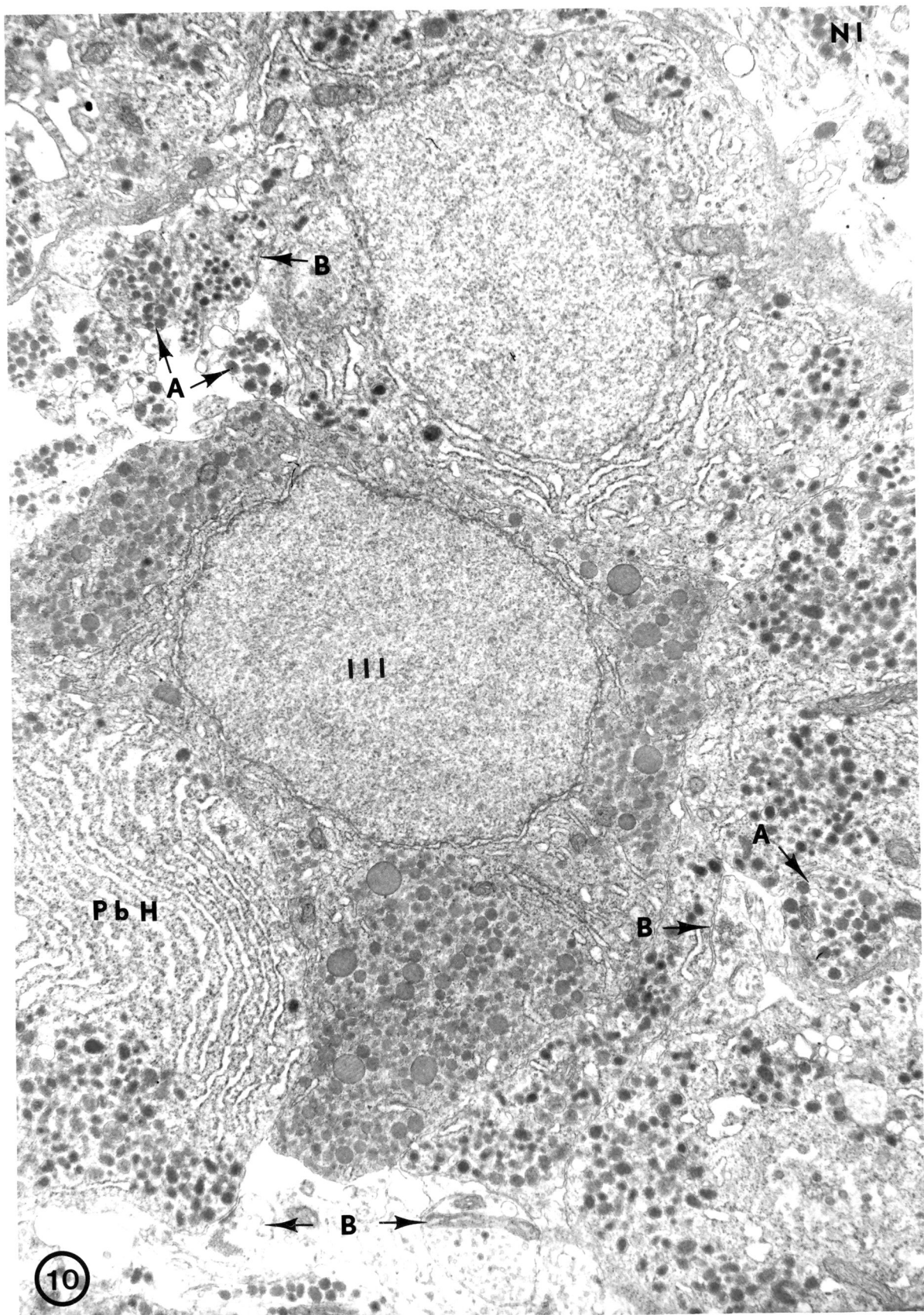


Figure 11. Rostral Pars Distalis. (RPD) A light staining chromophobe (LC) frequently found in this region. Note the long endoplasmic projection (arrows) that interdigitate with the prolactin cells (PROL) of this area. 4200X

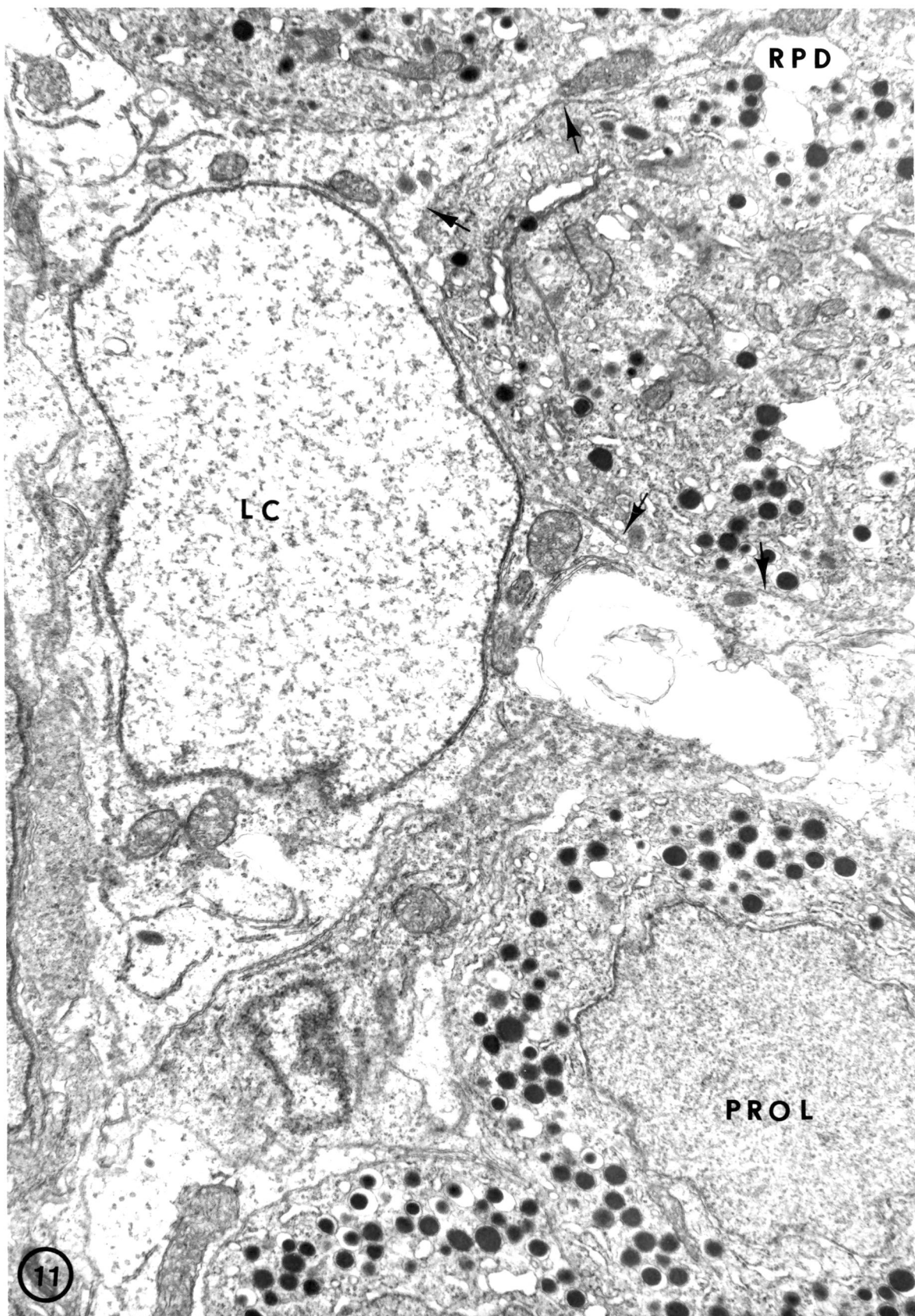
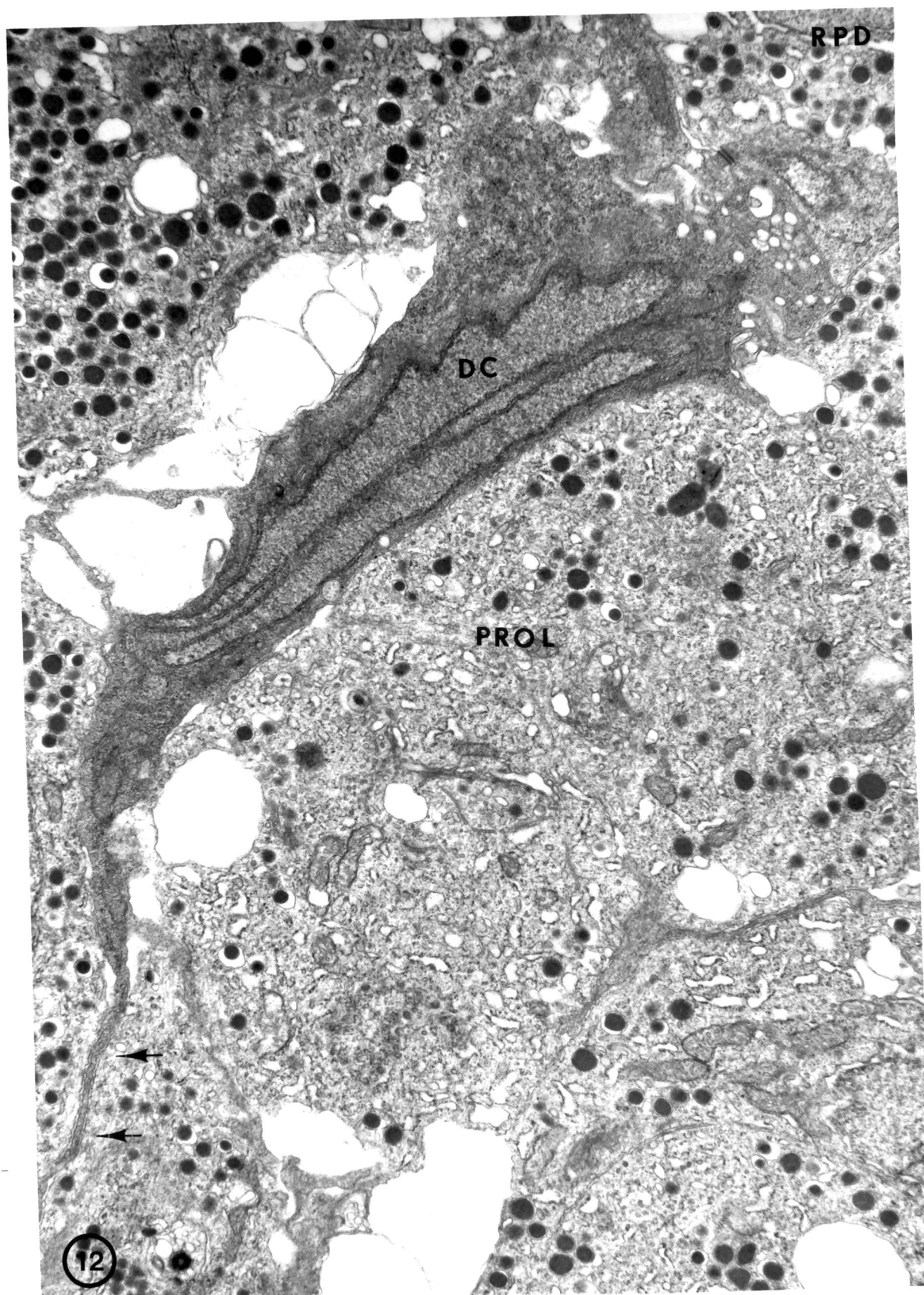


Figure 12. Rostral Pars Distalis. (RPD). A dark staining chromophobe (DC) frequently found in this region too. As in the case of the light chromophobe, the dark chromophobe sends long cytoplasmic projections far among the endocrine cells. 4200X.



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