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ULTRASTRUCTURE OF THE FROG PARS INTERMEDIA AND ITS RELATIONSHIP TO HYPOTHALAMIC CONTROL

by

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This manuscript has been read and accepted for the University Committee in Biology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

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ERRATA

Page vi and thereafter in text: For 0s04 read OsO_4.

Page 65, Line 23: For Z. Zellenforsch. read Z. Zellforsch.

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INTRODUCTION

The pars intermedia of the pituitary has been studied less extensively than the anterior and neural lobes. The majority of work on the pituitary is done in mammals, in which functions of the pars intermedia are poorly understood. In "lower" vertebrates, such as fish and amphibians, it is generally agreed that the function of the pars intermedia is to produce a hormone which regulates dispersion of pigment in melanophores, thereby controlling background adaptation. The bullfrog (*Rana catesbeiana*) and the leopard frog (*Rana pipiens*) were selected for study of the pars intermedia for the following reasons:

(a) Studies of frog pituitary generally make use of the two above named species, so that valid comparisons with previous work are possible.

(b) Healthy larval and mature specimens are available in almost all seasons.

(c) Animals are easily maintained under laboratory conditions.

(d) There is considerable interest in pars intermedia function and control in the laboratory in which this work was done.

Ultrastructural studies in amphibia are as yet relatively rare. The electron microscope was used to study the pars intermedia of frogs under different conditions of background adaptation, with the expectation that the higher resolving power might reveal cellular differences which could be correlated with changes in physiological function.

REVIEW OF THE LITERATURE

A. BASIC FUNCTIONS OF THE PARS INTERMEDIA:

The ability of many cold-blooded vertebrates to modify their skin pigmentation is well known. In particular, anuran amphibians are able to control the state of their melanophores in response to changes in the coloration of the environment. Smith and Smith (1923) found that bovine pars intermedia-pars nervosa extract restored normal pigmentation to hypophysectomized frog tadpoles. Allen (1929-1930) observed that pituitary grafts from toads implanted to host tadpoles led to darkening of the hosts, and suggested that the intermediate lobe appeared to be the major source of the darkening effect. Atwell and Holley (1936) stated that frog or salamander tadpoles remained silvery after hypophysectomy at tailbud stages because of lack of the pars intermedia, but they metamorphosed if the anterior pituitary was present. Furthermore, Zondek and Krohn (1932a, 1932b) reported a hormonal substance in the intermediate lobe of the minnow and other vertebrates, including frog and man, which caused expansion of melanophores, and named it "intermedin".

The classic paper of Hogben and Slome (1931) described the dark and light adaptive responses of *Xenopus laevis* in great detail, and attributed the major darkening effect to hormone from the pars intermedia. They considered the lightening effect to be the result of a "W" (whitening) hormone from the pars tuberalis (a portion of the pituitary which lies along the infundibular stem). They suggested that the final state of the melanophores was the result of darkening and lightening factors in the blood. Soderwall and Steggerda (1938) and Steggerda and Soderwall (1939) also stated that removal of the pars tuberalis (by insertion of a hot needle through the parasphenoid bone) abolished the lightening response. The two hormone hypothesis was adopted by Waring and Landgrebe (1950), and was most recently reviewed by Landgrebe and Mitchell (1966). The view that a lightening hormone originates from the pars tuberalis is not believed tenable, however , in the light of a study by Atwell (1941), which showed that the portion of the pituitary studied by Hogben and Slome was in reality a part of the anterior lobe, and not the pars tuberalis. Their 1931 experiments most likely damaged the hypophyseal stalk. Such damage (see below) leads to darkening in amphibians and many fish. Very recent work by Bagnara (see review, 1966) reported the presence of a lightening factor (melatonin) from the pineal gland of frogs, and the whole question of dual hormonal control of anuran coloration was reopened. The problem remains unsettled, as far as pineal control of color change is concerned. The present author accepts the view of Parker (1948) and Etkin (1968) that major control of background adaptation changes in anurans is via variations in the blood level of the pars intermedia hormone, hereafter referred to as melanocyte-stimulating hormone, or MSH.

B. LIGHT MICROSCOPIC ANATOMY AND PHYSIOLOGY OF THE PARS INTERMEDIA:

The intermediate lobe of the amphibian pituitary is a narrow band of cells located between the anterior and neural lobes. Most of the cells are polygonal and appear fairly uniform in shape. One also finds a number of rounded cells with slender, ependymal-like processes (see Wingstrand, 1966). The entire gland is relatively avascular, with venous drainage located at the border area near the pars nervosa.

Light microscopic histochemical studies by Ortman (1954) revealed that amphibian pars intermedia cells are Periodic-Acid Schiff positive, particularly in neutral and light-background adapted frogs, but the reaction appeared absent from many animals which had been adapted to black backgrounds. Ortman also noted an increased intensity in phospholipid staining in cells from dark-background adapted *Rana pipiens*.

Cellular volume changes may also be associated with alterations in background. Florentin and Stutinski (1936) observed increased cellular volumes in the pars intermedia of frogs kept in darkness. H. Legait (1962, 1966) was able to obtain similar results using dark-background adapted frogs. Furthermore, Legait and Legait (1964) noted that among sixteen species of amphibians, a larger pars intermedia was observed in those animals able to change coloration more effectively in response to environmental conditions. It must be noted, however, that Metzutals (1957) was unable to find any changes in cellular volumes in frogs kept in the dark, as opposed to those kept in light. It is possible that variation in experimental conditions led to variability of results in the study of volume changes.

C. ELECTRON MICROSCOPIC STUDIES OF THE PARS INTERMEDIA:

Ultrastructural studies of the pars intermedia are relatively rare. Among various species it appears that two or three cell types are usually present, with at least one having the characteristics of secretory cells. Pawlikowski (1967) found two cell types in the rat pars intermedia: one with dense nucleus and transparent cytoplasm, and the other containing many osmiophilic granules and microvillous projections. The latter type, observed near border areas, was presumed to be a secretory cell. In the cat, Bargmann and co-workers (1967) observed two types of polygonal epithelial cells, as well as a palisade cell, which was believed to resemble glial cells. In an earlier study, Bargmann and co-workers (1957) found that the pars intermedia of the snake, *Tropidonotus natrix*, contained rounded or polygonal epithelial cells, and within them, endoplasmic reticulum organized in parallel arrays, as well as masses of osmiophilic granules near the basal membrane.

Among mammals, some stressful conditions may lead to changes in the "secretory" cells observed in the pars intermedia. Ziegler (1963) noted the presence of dark and light cells in the rat pars intermedia, and stated that in animals subjected to osmotic stress, the ergastoplasm and Golgi regions of the dark cells developed to a greater extent, as compared to organelles in non-stressed controls. Kobayashi (1965) was also able to correlate an increase in the rat intermedia cell Golgi zone vesicles with a stressful situation (injection of formalin). The cells also showed increased activity when animals were adrenalectomized, and the author suggested a relationship between the pars intermedia and the adrenals. It will be noted later that melanocyte-stimulating hormone (MSH) and adrenocorticotrophic hormone (ACTH) have some identical amino acid sequences, so that in mammals, pars intermedia cells may assume some corticotroph-like functions.

In other vertebrates, changes in physiological state (other than stress) may be correlated with changes in pars intermedia cells. Birds lack a definite pars intermedia, yet Tixier-Vidal (1965) discussed an MSH cell type present in the adenohypophysis of the duck. After permanent light or reserpine treatment, there was extensive development of the endoplasmic reticulum and Golgi regions of the cell. Conditions such as molting and new feather growth also produced intense "activity" of the MSH cell. In the toad pars intermedia, Iturriza (1964) found three zones of cells, two of which appeared to be active in polypeptide synthesis, while the third (near the neural lobe) was suggested to be a colloid storage area. Cohen (1964 - see Knowles, 1965) observed increased amounts of rough endoplasmic reticulum in the pars intermedia of *Xenopus laevis* kept in darkness. In an elasmobranch, *Scylliorhinus*, Knowles (1965) described a pars intermedia cell which appeared polarized: one end contained rough ER (endoplasmic reticulum), and was presumed to produce polypeptide, while the other end held many dense granules, and was designated the area of secretion storage and release. Vollrath (1966) observed three cell types in the pars intermedia of the eel, *Anguilla*. One of these showed changes in rough ER and Golgi zones which could be correlated with development and function of the melanophores. Another pars intermedia cell type showed some evidence of being involved in osmotic changes. It may be noted that pars intermedia cytology appears fairly similar among the varied species, suggesting that different responses to secretions have arisen within the target organs, rather than through changes within the endocrine tissue.

D. CONTROL OF PARS INTERMEDIA FUNCTION:

The production of MSH by the intermediate lobe is under inhibitory control from the brain. Houssay and co-authors (1935) found that lesions in the infundibulum of the frog led to hypofunction of the anterior pituitary, and also led to darkening of the skin. Etkin (1938) found that partial or complete destruction of the infundibulum in tadpoles of _Rana sylvatica_ led to intense darkening, and hypertrophy and hyperplasia of the pars intermedia cells. He interpreted the findings to indicate that the severed nerve tracts normally inhibit secretory function of the gland. Etkin (1941a) later observed that the pars intermedia in ectopic sites produced similar intensive darkening, and histological examination of the grafts showed cellular hypertrophy. Etkin and co-workers (1961) and Etkin (1962a) more recently reported similar findings in adult _Rana pipiens_ after hypothalamic lesions were made, or after pituitaries were grafted to the anterior chamber of the eye. Stoppani (1942) observed darkening after lesioning the infundibular lobes of the toad, _Bufo arenarum_, as did Guardabassi (1961) in _Xenopus laevis_ after removal of the hypothalamic lobes. Eakin and Harris (1951) were able to cause darkening in the tadpole of the treefrog, _Hyla regilla_, with adult pituitary grafts. In salamanders, Mazzi (1958) reported intensive darkening and hypertrophy of the pars intermedia after median eminence lesions, while very similar experiments by Mellinger (1963) in the dogfish led him to state that control of the elasmobranch pars intermedia is via neural inhibition from the hypothalamus.

Among mammals, Barrnett and Greep (1957) noted the presence of an enlarged pars intermedia in rats with infundibular stalk section. Bogdanove and Halmi (1953) made a closely related study, and stated that the rat pars intermedia cells appeared vacuolated, and showed clear mitotic figures. The rabbit pars intermedia was also observed to hypertrophy after stalk section (Campbell and Harris, 1957). Likewise, cultured rat (Anderson and Haymaker, 1935) and mouse (Geiling and Lewis, 1935) pituitary was observed to produce a melanophore-expanding principle, the source of which was the intermediate lobe cells. In addition, Siperstein and Greer (1956) found increased volume, and many mitoses, in mouse pars intermedia which was grafted to the anterior eye chamber. Despite the fact that functions of the pars intermedia may vary considerably among different vertebrate phyla, it appears that neural inhibition from the brain is a common source of control.

E. EMBRYOLOGIC INVESTIGATIONS OF PARS INTERMEDIA:

Studies of the development of the pars intermedia in amphibians serve to form a more complete picture of the physiology of the gland. Blount (1932) stated that onset of pituitary production of MSH (as determined by melanophore expansion) occurred at stage 41 or 42 (Harrison, unpublished observations) in the salamander embryo. Atwell (1937) determined that pituitary primordia from tailbud embryos of *Rana pipiens* or *Ambystoma punctatum* could lead to darkening after placement in an ectopic site, and stated that darkening indicated development of pars intermedia function. Kleinholz (1940) found positive MSH activity in extracts of *Rana pipiens* heads at tailbud stage; however, Etkin (1941b) observed that in *Xenopus*, onset of function of pituitary primordium grafts in hypophysectomized hosts occurred at about 26 hours of development (at 20^{o} C.). The primordium appeared determined for future development to pituitary at stage 17 (tailbud) in *Rana sylvatica* (Etkin, 1958). Thurmond (1967) recently pinpointed onset of function of the pars intermedia in *Hyla regilla* at stage 23-24 (Eakin, 1947), and preliminary studies done by the present author suggest that onset of intermedia function occurs at comparable stages in *Rana pipiens*. It appears that production of MSH occurs relatively early in embryonic life, and that intermedia cells may function even before there is evidence for histological differentiation. Very recent ultrastructural observations in frogs (Smoller, 1966) and salamanders (Dent and Gupta, 1967) indicate growth of neural elements into the presumptive pars intermedia at the equivalents of stages 23-24 (Shumway, 1940), suggesting establishment of hypothalamic control at that time.

F. NATURE OF THE INTERMEDIATE LOBE HORMONE:

Careful biochemical studies of melanocyte-stimulating hormone have shown it to be a short-chain polypeptide (see Lee and Lerner, 1956, and Geschwind, 1966). Two major components, alpha and beta MSH, having thirteen and eighteen amino acids, respectively, have been isolated from mammalian pituitaries. Adrenocorticotrophic hormone (ACTH) is found to have as much as one percent intrinsic alpha-MSH activity (Lee and Lerner, 1956), and Geschwind notes in his review that the entire alpha-MSH sequence of thirteen amino acids is the N-terminal chain of ACTH. Although most biochemical studies have been done on mammalian material, Burgers (1960) reports that with electrophoretic methods, three MSH components could be isolated from the pituitary of the bullfrog and lizard, as well as from ovine, bovine and porcine preparations. However, electrophoretic mobilities of the various components were not the same for the different species. Harris (1966) suggested that substitutions on the MSH chain indicate that essential features for melanophore-stimulating activity are within positions 4-10 on the alpha chain. He noted that complete synthesis of the active principle has been accomplished in the laboratory. The latter author further stated that species variability in MSH arises from the presence of different amino acids on either side of the active heptapeptide.

G. IN VITRO STUDIES OF MELANOPHORES:

Cultured amphibian melanophores are found to respond to melanocyte-stimulating hormones and to various pharmacological agents in a manner close to that of melanophores in vivo. Zimmerman and Dalton (1961) observed that cultured Ambystoma xanthophores and melanophores expanded (i. e. there was movement of pigment away from the center of the cell) in the presence of ACTH or acetylcholine, while the catecholamines epinephrine and norepinephrine in the culture medium led to local aggregation of pigment and decreased cell size. Results were similar to findings in vivo. Novales and Novales (1965) found that hypotonicity produced melanophore expansion (which mimics MSH) while hypertonic media led to contraction. The action of MSH was also found to be calcium dependent. The same authors (1966) have recently reviewed many findings on cultured frog melanophores. Electron micrographs revealed an increased cell perimeter and the appearance of melanin-filled processes on the surface of the melanocyte after MSH treatment. Administration of epinephrine or the pineal hormone melatonin led to withdrawal of the processes and a decrease in complexity of the cell outline. The authors suggested that MSH may act on the cell by promoting sodium uptake, which in turn could produce changes in tonicity, thereby altering cell shape. They did not exclude the possibility of direct action of MSH on the cell membranes.

H. HYPOTHALAMIC RELATIONSHIPS TO THE PARS INTERMEDIA:

1. Chemical releasing and inhibitory factors:-

Attention has been focused for some time on chemical factors from the brain controlling the adenohypophysis. The vast literature on hypothalamic control of anterior pituitary function by chemical factors transported via the hypophyseal portal blood vessels will not be reviewed here, but the reader is referred to Volume I of Harris and Donovan (1966), and to a brief review by Knigge (1967). The latter author noted that ultrastructural studies of an area of median eminence and portal vessels reveal endothelial pores in capillary walls and microvillous projections from the neural area. He suggested that the cytological picture indicated release of substances in molecular form from neural areas into the capillary spaces.

As concerns MSH, Talesnik and Orias (1965) isolated an MSH-releasing factor from extracts of rat hypophyseal stalk and median eminence. The factor was shown to decrease MSH content from the pars intermedia, and was not an effect of catecholamines or cerebral cortex extract.

Investigators from the same laboratory group later reported (Dhariwal, et al, 1966 and Talesnik, et al, 1966) purification of the releasing factor, and localization of the factor primarily in the paraventricular nucleus and median eminence areas of the hypothalamus. A more recent report (Talesnik and Tomatis, 1967) stated that extracts of stalk-median eminence from toads did not give a releasing effect, and indeed, prevented the decrease in pituitary MSH levels if given simultaneously with rat extracts. The above findings appear somewhat in conflict with the hypothesis of hypothalamic inhibition of pars intermedia function, but the authors (Talesnik and Tomatis, 1967) suggested that at least in toads, there may be more than one controlling factor for MSH release.

In contrast to studies indicating isolation of MSH releasing factor(s), several laboratories have independently isolated MSH release-inhibiting factors from the brain. Kastin (1965) reported an increase in MSH activity of rat pituitary after in vitro incubation with hypothalamic extract. Kastin and Schally (1966a, 1966b, 1966c) found similar results in vivo with cold hypothalamic extracts, whereas with extracts prepared at room temperature (methods similar to those used by Talesnik and others, and not considered proper by Kastin and co-workers) a releasing effect was obtained. The authors suggested that the latter effect was an artifact, or that both releasing and inhibitory factors exist. The inhibiting factor obtained also lightened frogs previously darkened by hypothalamic lesions. More recent reports (Kastin and Schally, 1967a, 1967b) presented evidence for MSH release-inhibiting factors from hypothalami of various vertebrates, including frog, rabbit, sheep, pig, and man.

The results above have dealt generally with release of MSH from rat or mouse pituitaries. Ralph and Sampath (1965, 1966) demonstrated inhibition of MSH release from frog (Rana pipiens) pituitary by hypothalamic extract from frog or rat. In contrast to the results of Kastin, Schally and others, Ralph and Sampath could not demonstrate the effect in rat pituitary. Brinkley and Bercu (1965) and Bercu and Brinkley (1967) independently obtained reults comparable to those of Ralph and Sampath. No significant differences were found in extracts from dark or light adapted frogs, and Bercu and Brinkley suggest that inhibitory activity is attributed to catecholamine activity of the material (see further discussions below).

Hypothalami may also contain melanocyte-stimulating hormone-like properties. Guillemin and co-authors (1962) and Schally, et al, (1962) reported MSH activity in extracts of dog and pig hypothalamus, while Ralph and Peyton (1966) localized similar activity in the hypothalamus of Rana pipiens. Thurmond (1967) observed chromatophorotrophic activity in amphibian (frog, salamander) embryonic hypothalamus after its transplantation to young larvae. He stated that such activity could be separated from true hypophyseal MSH activity. Responses of the grafts disappeared within a few days.

The presence of MSH-releasing factors, as discussed above, may be an artifact of extract preparations. It seems more likely, in view of fairly conclusive evidence for inhibitory control of the pars intermedia, that any chemical factors from the brain are inhibitory. However, the existence of MSH release-inhibiting factors, and hypothalamic MSH-like material in highly purified extracts, in experimental situations, may not represent the true picture of intermedia control. As noted earlier, the pars intermedia of anurans is relatively avascular, and transport of humoral factors may therefore be difficult.

2. Nervous elements in the pars intermedia:

Studies of the pars intermedia of intact specimens strongly suggest that control is mediated by nerve fibers of hypothalamic origin, rather than via diffusible chemical factors. Production of neurohypophyseal hormones in the hypothalamic supraoptic and paraventricular nuclei of mammals, and transport of the hormonal material via axons to the pars nervosa are findings which form the basis for modern neuroendocrinology, and the reader is referred to the recent text of Harris and Donovan (1966) and to the work of E. and B. Scharrer (see review text, 1963). Nerve fibers which transport hormonal material in addition to transmitting impulses are termed neurosecretory. In this context, it may be noted that Dawson (1953), using an aldehyde fuchsin technique, found histological evidence for penetration of neurosecretory fibers from the neural lobe into the frog pars intermedia, although Nayar and Pandalai (1963) did not observe any nerve fibers in silver-stained preparations of lizard pars intermedia. Etkin (1962b) observed a "pileup" of neurosecretory material (positive staining with Halmi's aldehyde fuchsin technique) at the proximal end of the cut in stalk-sectioned frogs, and concluded that such material (usually not observed in intact preparations) could enter the pars intermedia without detection by histological means. Nerve fibers have definitely been observed with the light microscope in the pars intermedia of cattle (Ribas-Mujal, 1958), elasmobranchs (Meurling, 1963), and teleost fish (Follenius, 1965). Voitkevitch and Soboleva (1962) located a few nerve fibers and some neurosecretory granules in the pars intermedia of frogs, but stated that "activation" of the pars intermedia appeared to depend upon production of neurosecretory material by the preoptic nucleus. However, in a later paper (Voitkevitch, 1963), it was stated that high activity of neurosecretory neurons inhibits the pars intermedia, while cuts in the preoptico-hypophyseal tract lead to darkening. Voitkevitch noted that neural control of the gland appeared to be independent of any control exercised by the hypophyseal portal vessels. Jorgensen and Larsen (1963) demonstrated that the ability of _Xenopus_, _Bufo_, and _Ambystoma_ to adapt to dark and light backgrounds was lost after denervation of the neuro-intermediate lobe, and lightening ability was only regained after at least a month postoperatively, and often longer. Their conclusion was that nerves must regrow and contact pars intermedia cells before inhibitory function could be regained.

Dierickx has made many studies of gonadotrophic controlling centers in the hypothalamus, and recently investigated the question of neurosecretory control of pars intermedia function. His 1965 study showed that incomplete removal of the preoptic nucleus in frogs (equivalent to the neurosecretory nuclei of mammalian hypothalamus) did not lead to melanophore expansion, although gonadotrophic function was impaired, and later (1967) noted that all aldehyde fuchsin positive fibers originate from the preoptic nucleus. He concluded that function of the pars intermedia appears controlled by other, non-neurosecretory fibers. In contrast, ultrastructural studies of the fish neurointermediate lobe reveal that in teleosts (Follenius and Porte, 1962), neurosecretory-type fibers innervate cells, while in elasmobranchs, Mellinger (1962), Polenov and Belenky (1965) and Knowles (1965) independently observed several types of nerve fibers, including neurosecretory types, among neurointermediate cells. However, the neural and intermediate lobes of many fish are intermingled, rather than existing as separate entities, as in amphibia and many other vertebrates, so that the presence of neurosecretory elements among glandular cells is not too surprising. Neuroglandular relationships in anuran pars intermedia will be discussed in greater detail below.

It is generally concluded that amphibian pars intermedia function is controlled by neurons from some undetermined area of the hypothalamus. A recently developed fluorescent histochemical technique for localization of catecholamines and related compounds (Falck and co-workers, 1962) has been used to great advantage on nerve terminals believed to contain catecholamines. Fuxe (1964) stated that such compounds (specifically, epinephrine, norepinephrine and dopamine) can be observed in the mammalian median eminence and infundibular lobes. Of more immediate importance, Enemar and Falck (1965) observed characteristic fluorescence in the nerve plexus of the pars intermedia of the frog, Rana temporaria, while Dahlstom and Fuxe (1966) found fluorescent fibers in the pars intermedia of various mammals, including the monkey, rabbit, cat, dog, and three rodent species. Furthermore, use of the drug reserpine, which depletes cells and nerves of catecholamines, and darkens frogs (see Scott and Nading, 1961, and Khazan and Sulman, 1961) led to a loss of fluorescence in the fibers entering the pars intermedia in rats (Smelik, 1966) and in toads (Enemar and co-workers, 1967). It appears that adrenergic nerve fibers are the predominant type found among pars intermedia cells in many vertebrates other than fish.

The present study, as mentioned earlier, was undertaken to examine the pars intermedia of anurans with changes in the physiological state of background adaptation. At this time it may be stated that the ultrastructural findings suggest a pattern of secretion during dark adaptation, and confirm the predominant appearance of catecholamine-type nerve fibers on pars intermedia cells. Observations on glands taken from frogs at various stages of adaptation permit the presentation of a hypothesis for the means by which the hypothalamus controls pars intermedia function.

MATERIALS AND METHODS

A. PRELIMINARY EXPERIMENTAL METHODS:

Two species were used for the study of pars intermedia cytology: the bullfrog, *Rana catesbeiana*, and the leopard frog, *Rana pipiens*. Bullfrog premetamorphic larvae and young froglets were obtained from a Westchester, New York, pond in the spring, summer and early fall, 1966, while the adult leopard frogs were purchased from a Vermont supplier. *Rana pipiens* premetamorphic and prometamorphic larvae were raised in the laboratory from embryos secured by induced ovulation. All animals were kept in one-tenth physiological saline solution, and containers were placed on a cold water table (10°-16° Centigrade) with constant overhead fluorescent illumination in a room without windows. Larvae were fed canned spinach twice weekly. *Rana catesbeiana* froglets and *Rana pipiens* adults were not fed, except for four froglets, which were force-fed strained meat. No difference in pituitary cytology could be observed between fed and unfed animals, and it should be noted that frogs routinely survived and thrived for long periods, often several months, without feeding, if kept under the temperature conditions described above.

Rana catesbeiana larvae and froglets were adapted to dark or light backgrounds for three weeks to one month, while *Rana pipiens* tadpoles and adults were similarly adapted for periods of one or two weeks. The state of expansion of the melanophores in the dorsal tail fin (tadpoles) or web of foot (frogs) was determined by the Hogben and Slome (1931) melanophore index. Light adapted animals had an index of 1, while dark adapted ones had indices of 4.5-5.0. Bullfrogs were also fixed in transitional stages of background adaptation. For this, dark adapted animals were transferred to white dishes and observed frequently. When they began to lighten, with melanophore indices of 2-3.5, the pituitaries were removed and placed in fixative. Similarly, animals transferred from white to black backgrounds were sacrificed when their melanophores showed evidence of change (indices of 3-4). The records kept of transitional animals are summarized in Table I. The process of going from light to dark often occupied only a few hours, but varied, sometimes requiring overnight, or even longer. The reverse process (dark to light) also varied in terms of time required, but was generally completed in 12 to 24 hours.

Total numbers of animals used were as follows:-

Light adapted:

Rana catesbeiana - 8 froglets, 7 larvae
Rana pipiens - 6 adults, 5 larvae

Dark adapted:

Rana catesbeiana - 8 froglets, 8 larvae
Rana pipiens - 7 adults, 6 larvae

Points between the two extremes:

Rana catesbeiana - 10 froglets, 12 larvae.

B. TISSUE PREPARATION:

Animals were decapitated without anaesthetic, and the pituitary was quickly exposed by making two scissor cuts in the roof of the mouth. The parasphenoid bone was lifted off and drops of fixative were placed on the area (see Figure 1 for the procedure). Glands were quickly dissected out, freed of any attached calcified material (where possible without damage), and immersed in fresh fixative. Two fixation procedures were employed:

(1) Pituitaries were doubly fixed, using first glutaraldehyde, then osmium tetroxide. The glutaraldehyde used was 1.5% (made from Fisher biological grade stock - 50%) in 0.05 M sodium cacodylate buffer, pH 6.8-7.2. Fixation was carried out at room temperature for three to four hours. Glands were then placed in cold 0.13 M cacodylate buffer (pH 7.0-7.2) to which 0.10 M sucrose was added (642 mg. sucrose per 20 cc. buffer) in order to maintain an osmolality close to that of the fixative (about 300 milliosmols). The blocks were left overnight in the refrigerator (about 4 C.), then secondarily fixed for one to two hours in cold 1% OsO_4 in 0.10 M phosphate buffer, pH 7.0-7.3. Phosphate buffer was prepared by mixing 0.10 M sodium orthophosphate ($Na_2HPO_4 7H_20$) with 0.10 M

potassium orthophosphate (KH_2PO_4) in the proportions 9.5: 0.5, and adding concentrated hydrochloric acid to obtain the desired pH. After secondary fixation, tissue was rinsed in phosphate buffer, dehydrated in an ascending ethanol series (50%-10 minutes; 70%-15 minutes; 95%-30 minutes or overnight; 100%-30 minutes), then cleared with propylene oxide for 30 minutes to one hour. Infiltration was begun with propylene oxide and Epon 812 (1:1) for one hour (or overnight at room temperature). Blocks were embedded in pure Epon 812 (see Pease, 1964, for preparation of Epon) in plastic BEEM capsules, and polymerization took place for two or three days in an oven at 60° C. One micron sections were cut with glass knives using a Porter-Blum MT-1 ultramicrotome or a LKB Ultrotome. These sections were floated onto a glass slide in 10% acetone, dried on a hotplate, and stained for a few seconds with toluidine blue (1% toluidine blue in 1% borax). Thin sections (gold to silver interference colors, about 800 mu thick, according to Peachey, 1958) were cut with a DuPont diamond knife on a LKB Ultrotome. These were picked up on 200 mesh uncoated copper grids. Sections were stained for 15 minutes in 0.25% uranyl acetate in 50% ethanol, rinsedin distilled water, then stained a second time, in Reynold's (1963) lead citrate, for five minutes and rinsed again. Grids were examined in an RCA-EMU-3G electron microscope operating at 100 KV.

(2) Glands were singly fixed, utilizing 1% OsO_4 in 0.10 M phosphate buffer at pH 7.0-7.2, for one to two hours in the refrigerator (4° C.). Blocks were then dehydrated and embedded in the same manner as described above. (This second method was found generally to be less satisfactory as far as preservation of certain inclusions and membranes was concerned).

(3) An additional procedure was carried out in order to extract lipid material from pituitaries (the procedure was desirable because of the presence of inclusions in dark adapted animals which were thought to be lipids). Pituitaries from dark adapted bullfrogs were fixed in 1.5% glutaraldehyde in cacodylate buffer as described above. After an overnight rinse in cold 0.13 M cacodylate buffer, blocks were placed in absolute acetone for 5.5 hours in the refrigerator (4° C.). Tissue was then secondarily fixed with OsO_4 and handled thereafter in the same way as all other blocks. The procedure is modified from Ashworth and co-workers (1966), who extracted lipids from rat liver by placing aldehyde-fixed material in either absolute acetone, 95% ethanol, or a mixture of methanol and chloroform, for six hours, prior to secondary fixation with OsO_4. Thin sections of pars intermedia prepared as described above were stained for one-half hour in alcoholic uranyl acetate, or for ten minutes in lead citrate.

Light micrographs were made of one micron toluidine blue stained sections of dark and light-adapted pituitaries. Photographs were taken with a Zeiss Ultraphot on Agfa Isopan IFF clear base film, and developed for five minutes in Kodak DK-50. Electron micrographs were taken on Kodak 2" x 10" film strips, and enlarged from original 2" x 2" negatives onto Kodak or Agfa photographic paper.

OBSERVATIONS

(1) Light microscopy:

The pars intermedia of larvae and metamorphosed animals in both species showed no differences. Observations from both types are, therefore, grouped together for the respective species.

Sections of Rana catesbeiana pars intermedia reveal that the gland of both light and dark adapted animals (Figures 2a, 2b) is composed of a band of cells, most of which have rounded or oval nuclei. A less common cell type, with an irregularly shaped, denser staining nucleus, is also found. At the border zone between pars intermedia and pars nervosa, there are fewer nuclei, and long cellular processes are observed. All glands from dark adapted bullfrogs are characterized at the light microscopic level by the presence of large (1-2 microns), densely staining droplets in the peripheral region adjacent to the neural lobe. The droplets are occasionally found in deeper parts of the pars intermedia. They are not observed in glands from light adapted animals. However, one finds them in the pars intermedia taken from bullfrogs in transitional stages between light and dark extremes.

The pars intermedia of Rana pipiens appears essentially similar to that of the bullfrog (see Figure 2c). However, glands from dark and light adapted animals are generally not separable by any distinguishing characteristics. The figure shown is a one micron section of a Rana pipiens pars intermedia. In such sections, one observed large droplets rarely or not at all, and where found, they were located near the border with the pars nervosa in dark adapted specimens.

(2) Electron microscopy:

Observations from the two species at different adaptive states are described separately, and are summarized in Table II.

a. Light adapted bullfrogs:

The majority of pars intermedia cells from light adapted animals exhibit rounded or ovoid nuclei, as mentioned above. These cells, designated Type I, appear to be secretory. Their cytoplasm is filled with membrane-bounded granules (2,000-3,000 Å) of varied shapes and densities. The granules range in density from very dense (black in appearance) to fairly light (light gray in appearance). Some granules which are intermediate may be found, but they appear relatively rare. Figures 3 through 7 illustrate the light adapted Rana catesbeiana pars intermedia. Cytoplasmic granules are very poorly preserved in tissue fixed with osmium tetroxide alone, and so most figures so prepared show few or none of them. Cytoplasmic details of Type I cells:- The rough endoplasmic reticulum of "secretory-type" cells of light adapted bullfrogs is usually present as parallel arrays, but may also be observed as isolated sacs. Slight distension of the endoplasmic reticulum (ER) is observed in some Type I cells, with occasional fine filamentous material within cisternae (see Figure 6). Figure 5 illustrates the Golgi region of a Type I cell. In the light adapted animal, Golgi membranes are slightly distended. Electron-dense, membrane-bounded granules are associated with the membranes, and in some specimens, the appearance of the granules suggests that they may be pinching off from flattened Golgi cisterns. The sizes and shapes of all membrane-bounded granules, regardless of density, suggest that they are related to one another. It is possible that the less dense granules are derived from granules associated with the Golgi zone.

b. Dark adapted bullfrogs:

There are three major distinguishing characteristics in the cytoplasm of dark adapted bullfrog pars intermedia Type I cells. The first is an increase in amount of rough endoplasmic reticulum, which appears as whorls or expanded parallel cisternae, as illustrated in Figures 8, 10, 12, and 13. There is fine filamentous material within intracisternal spaces. Cytoplasmic granules which appear identical to those in light adapted Type I cells are also present in dark adapted glands. Some granules may be located between expanded cisterns of rough ER (Figure 11).

A second characteristic is the presence of large

(1-2 microns) electron-dense droplets (see Figures 9, 12, 14, and 15). They are present in "secretory" cell processes near the neural lobe border, or more rarely, in deeper regions. The droplets are assumed to be the same structures observed near the border area in light microscope sections. The electron microscope shows them to be located within expanded cisternae of rough ER, and to be surrounded by fine filamentous material with which they may be continuous (Figure 30). The size, shape, and extreme density of the intracisternal droplets at first were suggestive of lipid composition. Accordingly, the lipid extraction procedure modified from Ashworth and co-workers (1966) was applied to four long-term dark adapted pituitaries. Figure 15 shows that the droplets remain, although cell membranes, mitochondria, and other membrane structures are not intact. An additional finding is that the cytoplasmic granules remain, although their surrounding membranes are broken or absent. While the use of acetone does not guarantee the removal of all lipid or lipid-like material from the droplets or elsewhere, it is strongly suggested that the droplets (and cytoplasmic granules) are not primarily composed of lipid. The possibility of a polypeptide nature for both structures will be discussed in detail later.

The third distinguishing characteristic of dark adapted bullfrog pars intermedia is an increase in numbers of dense granules associated with the Golgi region. Dense material is observed within flattened cisterns, and also appears to pinch off from expanded ends of Golgi cisterns. In addition, small vesicles are present in the cytoplasm of Type I cells in regions between Golgi zones and whorls or expanded areas of rough endoplasmic reticulum (see Figures 11, 12, and 13).

The second cell type, designated Type II, is observed in the pars intermedia of all specimens. It has a more irregularly shaped nucleus than the Type I cell, appears more electron dense, and exhibits long, slender cytoplasmic processes which extend between Type I cells, and are often observed near the borders of the pars intermedia (see Figures 4 and 27). The dense, scanty cytoplasm of the Type II cell does not contain "secretory-type" granules. This cell type does not appear to vary with changes in the adaptive state of the animal.

c. Bullfrogs undergoing darkening (melanophore indices 3-4):

Type I cells from bullfrog tadpoles and froglets in the process of darkening after light adaptation appear somewhat intermediate between the two extremes described above. Some cells within one pituitary show partially expanded cisternae of rough ER, with occasional whorl formation (Figure 18). In other cells, the rough ER appears relatively flattened (Figures 16 and 17).

Large, dense intracisternal droplets are present in varying numbers in darkening animals. They are located near the pars nervosa, or in more proximal Type I cell processes (Figures 19 and 20).

Specimens undergoing darkening are releasing MSH into the blood, and so the border areas of the pars intermedia were studied for possible evidence of release. Only one extracellular granule was found (Figure 24), although many sections were searched, and that granule may have been artificially displaced. Other sections (Figure 23) showed cytoplasmic projections from pars intermedia cells, as well as "blebbing" of endothelial cell regions. However, such observations were not believed to present clearcut evidence for release of material. Types of release mechanisms which may predominate in the pars intermedia will be considered in the Discussion.

d. Bullfrogs undergoing lightening (melanophore indices 2-3.5):

The cytology of Type I cells of bullfrogs placed on light backgrounds after dark adaptation may also be somewhat intermediate between that of animals at either extreme. However, in this group, many cells appear quite similar to those observed in dark adapted specimens. One frequently observes whorls and expanded rough ER cisternae (Figures 25 and 26) and numerous dense granules associated with the Golgi zone (Figures 27, 28, and 29). Large, dense droplets are present in every animal in this group, often close to the border between the pars intermedia and pars nervosa (Figure 30).

It should be emphasized that neither group of transitional bullfrogs show characteristics as extreme and extensive as dark adapted animals. Within the same pars intermedia, cells range from having extensive ER and Golgi zone granules in abundance to those with less conspicuous changes.

e. Light adapted Rana pipiens:

The electron microscope reveals that the pars intermedia cells of this species appear very similar to those of bullfrogs. Cell types are accordingly designated Type I and Type II, as they are for the bullfrog. In light adapted animals, the cytology of "secretory-type" cells is basically the same as in the bullfrog (see Figures 32 and 33). Rough ER is present as flattened cisternae or slightly dilated sacs (Figure 34). The cytoplasm is filled with membrane-bounded granules (2,000-3,000 Å) which have shapes and densities very much like those in bullfrogs (Figure 35). The Golgi region, essentially the same in appearance as in light adapted bullfrogs, has some dense material associated with its membranes and cisterns.

f. Dark adapted Rana pipiens:

The whorls and expanded areas of rough ER observed in bullfrogs are also found in Rana pipiens

(Figures 37, 38, and 39). Numerous dense, membrane-bounded granules are present near Golgi membranes, and there is also dense material within the cisterns (Figure 37). Large, dense intracisternal droplets, which were consistently observed and quite numerous in dark adapted bullfrog pars intermedia, are only rarely found in the pars intermedia of _Rana pipiens_. The few droplets which were observed (Figures 40 and 41) were located after careful searching of many sections. They appear to be quite similar to those in bullfrog glands. Preliminary quantitative studies of relative area occupied by cytoplasmic granules in _Rana pipiens_ adults (see Table III for technique and results) suggest that there is a decrease in relative amounts of granules in dark adapted animals as opposed to light adapted ones. However, the results might reflect changes in cell volume or movement of granules within the cells.

Type II cells in light and dark adapted _Rana pipiens_ appear very similar, a finding which is consistent with that of bullfrog pituitaries. Nuclei of Type II cells are reminiscent of those in bullfrogs, although the _Rana pipiens_ cells may app-ar somewhat less electron-dense (Figure 32). Slender processes of Type II cells run between Type I cells, often extending to border areas.

g. Cilia in the pars intermedia:

Cilia are observed in Type I cells of the pars intermedia from both species. A longitudinal section of a basal body region of a cilium with a rootlet is shown in Figure 42 from _Rana pipiens_. The cilia appear to have a characteristic 9+2 pattern of fibrils, although some may be 9+0. The functional significance of cilia in the pituitary gland is uncertain, and will be discussed further below.

h. Nerve fibers in the pars intermedia:

The pars intermedia of both species contains unmyelinated nerve fibers and terminals (for clear examples, see Figures 6, 7, 34, and 42). At higher magnifications, fibers are observed running among cells of both types, and terminating upon Type I cells in what appears to be synaptic contact (as described by DeRobertis, 1959; 1967). Where terminals occur, there is a pile up of small, clear vesicles (200-300 Å) against a thickened membrane, (see also Figures 21, 22, 31, and 43). Larger vesicles (600-1,000 Å) characterized by a dense core which is separated from a limiting membrane by a clear space, are also present in some terminals, as well as in fibers which are coursing through an area. In _Rana pipiens_, one terminal is observed to contain larger vesicles (1,000-1,500 Å) as well as synaptic-type vesicles (Figure 36). It closely approximates the "peptidergic" fibers described by Bargmann and co-workers (1967) in the cat pars intermedia.

Fibers containing elementary granules of classical neurosecretory type (1,000-2,000 Å), but without synaptic-type vesicles, appear to penetrate from the pars nervosa, and closely approach pars intermedia cell processes (Figure 14). Small-granule nerve fibers are observed throughout the pars intermedia, but fibers containing neurosecretory-type granules are only found near the neural lobe border.

DISCUSSION

The evidence presented suggests that the light and dark adapted conditions, as well as transitional states of background adaptation in frogs, may be correlated with specific cytological details in pars intermedia cells.

A. TYPE I CELL CHANGES:

The characteristic whorls and expansions of rough ER in dark adapted animals is consistent with increased polypeptide synthesis, probably involving formation of MSH or a precursor material. Kurosumi (1961), in an extensive review paper of the secretion mechanism, states that rough ER appears to be actively involved in synthesis of protein. He pointed out that often there may also be an increased number of smooth-surfaced vesicles in cells with heightened secretory activity. Such vesicles were found in dark adapted frog pars intermedia. The increased numbers of dense granules in the Golgi region also is consistent with augmented polypeptide synthesis during dark adaptation. It is therefore suggested that pars intermedia cells synthesize material (probably polypeptide) on the rough ER, presumably on the ribosomes, and then transport it to the Golgi region, where it "condenses" as very dense cytoplasmic granules. Iturriza (1964) observed similar cytoplasmic details in the pars intermedia of winter toads. He did not determine the state of background adaptation of the toads; however, such hibernating animals (at least in *Rana pipiens*) are usually fairly dark. It is probable that the state of the pars intermedia studied by Iturriza corresponds in part to that of dark adapted frogs. Cohen (1967) recently reported changes in ER in dark adapted *Xenopus laevis* similar to those found here. Ito (personal communication) noted an augmentation, with some formation of whorls, of rough endoplasmic reticulum, in specimens of *Rana nigromaculata* which had received hypothalamic lesions, with subsequent darkening. Masur (personal communication) observed increased amounts of rough ER and Golgi zone granules in the grafted pars intermedia of the salamander, *Diemyctylis viridescens*, as did the present author in preliminary electron microscopic studies of larval *Rana pipiens* pituitary grafts. Grafted glands, as noted earlier, like those of animals with hypothalamic lesions, are hyperactive in MSH secretion.

B. NATURE OF CYTOPLASMIC GRANULES:

The cytoplasmic granules which are so prominent in all animals are believed to represent storage of one or more forms of hormone. One possibility is that the dense and less dense granules are the several known types of MSH (see Geschwind, 1966). However, since transitional forms of granules were infrequently observed in this study, the granules may be different phases of stored hormonal material. Preliminary findings in the present study suggest that relative numbers of granules may decrease somewhat in dark adapted Rana pipiens, as compared to light adapted animals. Ortman (1956) was unable to find significant differences in MSH content of the pars intermedia of dark or light adapted Rana pipiens adults. The dark adapted gland of Rana esculenta has been found to have larger cell volumes (Legait, 1962) than light adapted ones. It is possible that relative density of granules may decrease in dark adaptation, although relative amounts of MSH in the two types may not change. Some studies of animals with hypothalamic lesions indicate depletion of stored MSH in the pars intermedia, which may be correlated with an observed decrease in numbers of the cytoplasmic granules. Kastin and Ross (1965) found that after lesions, there was a very significant decrease in MSH content of the pars intermedia of Rana pipiens. (It should be noted, however, that Voitkevitch, 1966, was not able to find any significant differences in MSH content in glands from animals with lesions, as compared to intact frogs.) Ito (personal communication) was able to correlate a decrease in MSH content of the pars intermedia in Rana nigromaculata with electron micrographs showing decreased cytoplasmic granules. Such correlation of granules with hormone content strengthens the hypothesis of a storage function for the granules. It is suggested that under physiological conditions of adaptation, demand for hormone may be met without excessive depletion of cytoplasmic stores, whereas in animals darkened by lesions, output of MSH may be so great as to deplete stores of hormone faster than synthesis can replace them.

C. DENSE INTRACISTERNAL DROPLETS:

The nature of the large intracisternal droplets observed in dark adapted and transitional bullfrogs is not clear. They do not appear to be lipid droplets (they remain after acetone treatment, and are located within cisterns of rough ER), and may be of a polypeptide nature (see Iturriza and Koch, 1964a, below). Dense intracisternal granules were observed by Palade (1956) in the guinea pig pancreas, and in the dog pancreas by Ichikawa (1964). Palade suggested that the rough ER was involved in direct production and/or segretation of material comprising the granules. Kurosumi (1961) pointed out that "half-made" protein secretion, formed on ribosomes, may be somehow hindered in transport to the Golgi zone, and secretory granules may appear in ergastoplasmic cavities. The droplets observed in this study are believed to be formed in a manner similar to that proposed for the pancreatic granules.

With the light microscope, Ortman (1956) observed what he considered to be intercellular vesicles near the border of the transverse vein in Rana pipiens, but did not correlate their appearance with a particular adaptive state. He suggested that they formed by fusion of smaller granules which could be observed within pars intermedia cells. Nayar and Pandalai (1963) found that the pars intermedia of dark adapted lizards (Calotes versicolor) showed large amounts of acidophilic colloid droplets near capillaries and marginal blood vessels. Animals transferred to light backgrounds showed a decreased amount of colloid. Legait (1962) noted large Periodic-Acid Schiff (P. A. S.) positive inclusions in the area of the intermediate lobe next to the neural lobe in Rana esculenta, and stated that they tended to decrease in number in light adapted animals, as compared to dark adapted ones.

Using electron microscopy, Iturriza (1964) found inclusions which appeared identical to the droplets here described, and suggested that they contained stored MSH. (Iturriza did not see smaller cytoplasmic granules in the toad pituitary, and it is the opinion of the present author that he did not because he used only osmium tetroxide for fixation. In the present study, cytoplasmic granules were never preserved to any extent with osmium alone). Additional studies with the light microscope (Iturriza and Koch, 1964a) indicated localization of some alpha amino acid components of MSH within the inclusions, which were termed "colloid vesicles". The authors, in another publication (1964b) found an increase in numbers of "colloid vesicles" after administration of the drug lysergic acid diethylamide (LSD), which causes lightening in the toad *Bufo arenarum*, and in *Xenopus laevis* (Burgers, et al, 1958). They interpreted the finding to favor the hypothesis of MSH storage function for the inclusions. LSD is a serotonin antagonist, and is presumed in the above case to act at some level of neural control of synthesis and/or release of MSH (see Iturriza, 1965). It has also been shown, however, that LSD causes darkening in the frog, *Rana temporaria*, and in many teleosts (see Burgers, 1966), so that the effects of the drug may be species specific, and not necessarily interpretable in terms of hormone storage. In the present investigation, intracisternal droplets were not observed in light adapted animals, and were only infrequently found in dark adapted *Rana pipiens*. No evidence for release of droplets was found, and it is therefore suggested that they represent material formed on the rough ER which directly condenses within ER cisternae during periods of increased synthesis and release. Their eventual disappearance in glands after light adaptation may result from dissolution or other alteration within the cisternal spaces. In summary, it is suggested that hormone is synthesized in the rough ER, normally packaged in granules (2,000-3,000 Å) in the Golgi region, and stored in the cytoplasm. When synthetic activity is very high in the normal bullfrog, and in some cells of <u>Rana pipiens</u>, material may condense directly within cisterns as dense droplets.

D. TYPE II CELLS:

The less common cell type does not show changes in either species with alterations in background adaptation. Kobayashi (1965) observed similar cells in the rat pars intermedia, and believed them to be a degenerative type, while Bargmann and co-workers (1967) suggested that such cells in the cat pars intermedia resembled glial cells. Type II cells may be similar to cells of the latter type, since processes from them run between many other cells, and often reach to the edges of the gland. Their morphology appears to also correspond to that of "stellate" cells in the pars distalis of the dog (Kagayama, 1965), which are believed to be of a "supportive or sustentacular nature".

E. MSH RELEASE:

There were no consistent observations indicating release of granular material from pars intermedia cells of dark adapted or transitional animals. The only extracellular granule found (see above) was believed to be artificially displaced. Some microvillous projections were observed near capillaries in light-to-dark transitional bullfrogs, but they were not overly conspicuous. There was no budding of cytoplasm or fusion of granules with plasma membranes, such as observed by Weiss (1965) in the anterior pituitary of the fish, Xiphophorous, and observed by Smith and Farquhar (1966) in mammotrophic cells of the anterior pituitary of lactating rats. Salazar and Peterson (1964) also showed evidence of "microapocrine" secretion in anterior pituitary, with breaking off of blebs containing secretory granules. Masur (personal communication) was unable to find morphological evidence for granule release from the pars intermedia cells of pituitary grafts in the salamander, although she was able to find numerous examples of granule release from anterior lobe prolactin cells. Kurosumi (1961) suggested that some secretory cells could release products by morphologically undetectable means, presumably at a molecular level of transport across membranes. Dark adapted and transitional frogs changing from light to dark are releasing MSH, so that one must assume that there is molecular release of hormone from the pars intermedia.

F. CILIA IN THE PARS INTERMEDIA:

Cilia and ciliary rootlets (Figure 28) were observed arising within pars intermedia secretory cells in both species of frogs. Embryologically, the pars intermedia arises from an ectodermal derivative, the hypophyseal anlage, which migrates into the embryo from the exterior until it makes contact with the diencephalon. Since ectodermal derivatives often possess cilia, their presence is not too surprising. Barnes (1961) observed 9+0 cilia in secretory cells of the mouse pars distalis, and believed them to be sensory in function. She suggested that the 9+0 pattern was degenerate from a 9+2 pattern of fibrils. 9+0 cilia were also frequently observed in rabbit pars distalis (Salazar, 1963), and it was noted that only one cilium could be found in any particular cell. A more recent study of the rat adenohypophysis (Wheatley, 1967) revealed occasional cells containing two 9+0 cilia. Knowles (1965) noted the presence of 9+0 cilia as well as ciliary rootlets in the secretory cells of the neuro-intermediate lobe of the dogfish, and suggested that they might have a sensory function. Electron micrographs presented here show cilia which appear to be 9+2 (although some 9+0 may exist). Since they are observed mainly within secretory cells, often within deep parts of the pars intermedia, their functional significance (if any) remains unclear.

G. SIGNIFICANCE OF NERVE FIBERS:

The presence of nerve fibers and synaptic terminals containing small granules in the frog pars intermedia can be correlated with previous electron microscopic studies. Fibers and terminals containing small, clear vesicles and dense core vesicles have been found in the pars intermedia of elasmobranchs (Mellinger, 1963a, 1963b; Knowles, 1965), teleosts (Vollrath, 1967), the rat (Kobayashi, 1965), the cat (Bargmann, et al, 1967), the developing newt (Dent and Gupta, 1967), and the developing treefrog (Smoller, 1965), Cohen (1967) observed small-granule nerve fibers in Xenopus pars intermedia, but did not see synaptic-like terminals. Bargmann and co-authors also found a third type of terminal, termed "peptidergic", which contains both small, clear vesicles (200-300 Å) as well as granules in the size range of classical neurosecretory granules (1,000-2,000 Å). A fiber which can be described as "peptidergic" was observed in the pars intermedia of Rana pipiens, near the border with the neural lobe. In an elasmobranch, Knowles (1965) observed two types of nerve fibers in intimate contact with pars intermedia cells, and suggested that one type ("A" fibers, equivalent to the "peptidergic" fibers) appear to be classical neurosecretory fibers, while the other ("B" fibers) was monoaminergic in appearance. "A" fibers were believed to control synthesis, since they synapsed with the "synthetic" pole of the secretory cell (rough ER in parallel arrays was found at this pole), while the "B" fibers were suggested to control release of secretory material. Kurosumi and co-workers (1961) observed what might have been neurosecretory material within axons of the rat pars intermedia, while Zeigler (1963) stated that he definitely saw such material within axons in the rat intermediate lobe. Iturriza (1964) observed neurosecretory fibers from the neural lobe close to pars intermedia cells in the toad, Bufo arenarum, but did not find many fibers of any kind in deeper regions of the gland. In the present study, fibers containing granules in the size range of classical neurosecretion were present only very close to the border of the neural lobe (Cohen, 1967, made similar observations in Xenopus.) These may be interpreted as nervosa fibers which have "wandered" out of the lobe. Their significance is uncertain here, although their more regular occurrence in elasmobranchs, the cat and the rat suggests function. Attention is focused on the "B" type fibers, since only they are present throughout the frog pars intermedia.

Some recent histochemical and pharmacologic techniques utilized for localization of catecholamines were reviewed earlier. Nerve fibers in the "colloid vesicle" zone of the toad pars intermedia (Iturriza and Mestorino, 1965) were shown in a later, similar study to emit fluorescence characteristic of catecholamines (Enemar, et al, 1967). Iturriza (1966a, 1966b) also observed that the use of injected reserpine in toads depleted the pars intermedia of catecholamine-type fluorescence. Precise E/M localization of catecholamines within nerves is not entirely settled. Fuxe and co-authors (1966) believe activity is centered in the small, clear vesicles within nerve fibers. They state that no changes in granular (dense-core) vesicles is observed after use of reserpine or monoaminoxidase inhibitors in rat brain, and that the granular vesicles are present wherever histochemical means reveal catecholamines. However Hokfelt (1967) observed a depletion of granular vesicles after reserpine treatment of rat brain slices. Pellegrino de Iraldi and co-workers (1963) localized tritiated norepinephrine in the granular vesicles of rat anterior hypothalamus. Lenn (1967) recently obtained similar results with labelled norepinephrine injected into rat lateral ventricle, and also localized catecholamines in granular vesicles in vitro. The totality of evidence forms a consistent picture supporting the concept that the pars intermedia cells are under inhibitory control by monoaminergic nerves, which at the ultrastructural level appear as "B" fibers, containing small, clear synaptic-type vesicles (200-300 Å) and larger dense-core vesicles (600-1,000 Å) which may be storage sites for catecholamines or related compounds.

H. BULLFROGS IN TRANSITIONAL STAGES:

Findings suggest that many, but not all, cells of transitional bullfrogs appear active in synthesis. In dark-to-light animals, it is presumed that the level of hormone in the blood has been cut off sharply, in comparison to dark adapted animals, since the melanophores of the former group had begun to contract at the time of fixation. Glands were therefore fixed when under inhibition. It is hypothesized that inhibition of the pars intermedia first occurs in this species at the level of hormone release, and as unreleased hormone accumulates, synthesis is slowed down to the point at which one finds the cytological picture of a light adapted animal.

On the other hand, in animals changing from light-to-dark, hormone release must have begun shortly before fixation, since melanophores expand rapidly in response to a rise in blood MSH levels. The cytology of the pars intermedia of this group suggests that, at least in some cells, synthesis of new hormone begins promptly after initiation of hormone release. Figure 44 summarizes the hypothesis presented. By tying synthesis to the release mechanism, the pars intermedia may preserve a high titer of hormone in both dark and light adapted animals, as Ortman (1956) has found using assays of frog pituitary MSH. Ito and Suzuki (personal communication) observed that the drug, 2,3-dimercaptopropanol darkened light adapted frogs, but did not decrease MSH potency of the pars intermedia, whereas dark adapted animals became more intensely black with the drug, and showed a significant drop in MSH activity of the gland. The authors suggest that the drug had two effects: it increased release in already darkened frogs, and may have acted to store hormone in light adapted animals. One might explain the results with one hypothesis: that the drug caused MSH release in all animals, but dark adapted frogs, since they are continuously releasing hormone, are unable to maintain a synthetic rate which can preserve a constant level of MSH. Loss of hormone would, therefore, be detectable in them.

Dual nervous control of both MSH synthesis and release, as hypothesized by Knowles (1965) for the dogfish, does not appear to exist in the anurans studied here, since the only fibers which penetrate throughout the pars intermedia are small-granule types. Cohen (1967) suggested that the dual control may exist in Xenopus. His observations were very similar to those made here, however, in that neurosecretory-type endings were observed only near the neural lobe, and so one might interpret findings in Xenopus pars intermedia to suggest control by small-granule nerve fibers. Because it has been shown that the intermediate lobe of elasmobranchs is under inhibitory control from the brain (Mellinger, 1963), it is possible that the mechanism for control of hormone release is the same as for anurans, namely, inhibition by catecholamine fibers. The present findings do not support a hypothesis for neural control of synthesis in frogs. The work of Kastin and Ross (1965) and Ito indicates that loss of nerve supply (or administration of possible MSH releasing drugs) leads to so great a rate of release that synthesis is unable to keep pace, and there is depletion of cytoplasmic stores.

The means by which nerve fibers exercise control of cellular function of the pars intermedia is not clear. No differences were observed in contents of nerve fibers, regardless of adaptive states of the frogs. Micrographs did not indicate how monoamines directly influence endocrine cells, although the presence of many intact synapses suggests that control is mediated directly through them. According to Etkin (1968), inhibitory control of the gland must reach all cells to be effective, since a few uncontrolled cells can produce enough hormone to darken an animal. The synapses observed appear to be in sufficient numbers to exercise such control. Knowles (1965) suggested that contents of nerve fibers entered endocrine cells through gaps in membranes, but did make note of the possibility that such gaps could be artifacts. DeRobertis (1959) stated the now widely accepted view that the nervous action potential liberates a transmitter to the synaptic cleft (in axo-dendritic and myoneural junctions), transmitter diffuses across the cleft to the subsynaptic membrane, and then transmitter molecules attach to specific sites and lead to changes in permeability of various ions. A flow of current is thereby set up (synaptic potential) in the receptor area. In a later report (DeRobertis, 1967), it was noted that receptor properties are localized in junctional complexes, probably at the subsynaptic membrane. It is suggested that in the pars intermedia, release of transmitter at synapses in response to nervous impulses from the hypothalamus alters secretory cells, either at membranes or at the level of transport systems, so that release of MSH is halted. Buildup of hormone may then provide a negative feedback to the protein synthetic mechanism.

SUMMARY

The pars intermedia of larval and metamorphosed bullfrogs (*Rana catesbeiana*) and leopard frogs (*Rana pipiens*) placed under varying background conditions was studied by light and electron microscopy. Two cell types are found in all glands: Type I, or secretory cells, which possess fairly well-developed endoplasmic reticulum and Golgi regions, and Type II cells, which exhibit a dense nucleus with scanty cytoplasm. The latter cell type puts out long processes, and may resemble glial cells. Type II cells do not show variations, regardless of adaptation.

In light adapted animals, Type I cells show relatively flattened endoplasmic reticulum, and Golgi regions containing a few dense granules. The remainder of the cytoplasm is filled with membrane-bounded granules (2,000-3,000 Å) of varied shapes and densities. Such granules are present in every animal studied if glands are fixed with glutaraldehyde and osmium, but are lost with osmium fixation alone.

There are three major distinguishing characteristics in the cytoplasm of Type I cells from dark adapted animals. The first is the presence of whorls or expanded cisterns of rough endoplasmic reticulum. Fine filamentous material is present in intracisternal spaces. A second characteristic is an apparent increase in dense granules associated with the Golgi zone. Dense membrane-bounded granules are observed "pinching off" from Golgi membranes. The third characteristic (occurring mainly in bullfrogs) is the presence of large (1-2 u) dense droplets within expanded cisterns of rough endoplasmic reticulum. The changes observed in dark adapted animals as compared to light adapted ones strongly suggests that active protein synthesis is occurring, even to the point where material (the droplets) may directly condense in ER cisterns after synthesis. Release of granules and/or droplets from pars intermedia cells was not observed, and it is suggested that MSH is released into the blood in a molecular form rather than as visible material.

Nerve fibers and clear synaptic terminals containing small, clear vesicles (200-300 Å) and dense core vesicles (600-1,000 Å) were observed in the pars intermedia of all specimens. Synapses occurred directly upon Type I cells. Near the border zone of the pars intermedia and pars nervosa, fibers containing classical neurosecretory-type granules (1,000-2,000 Å) were found, but these did not occur in deeper regions. Based upon evidence from previous histochemical and pharmacological studies, it is hypothesized that the small-granule fibers contain catecholamines, and are the source of hypothalamic inhibitory control of the pars intermedia.

Bullfrogs in transition from dark to light or vice versa exhibit pars intermedia cytology somewhat intermediate between the two extremes. Some cells of light to dark animals show some expansion of endoplasmic reticulum and increased numbers of Golgi zone granules, while others have characteristics closer to those of light adaptation. Animals transferred from dark to light have many cells which appear very similar to those of dark adaptation. Glands from animals in the latter group were fixed while under inhibition, and it is suggested that neural control first inhibits at the hormone release level. In light to dark bullfrogs, hormone release is believed to occur rapidly, and is shortly followed by new synthesis. It is suggested that synthesis is controlled by a negative feedback: as unreleased hormone accumulates, production is slowed until one finds the cytological picture of a light adapted animal.

TABLE I. RECORDS OF CHANGES IN BACKGROUND ADAPTATION IN TRANSITIONAL *RANA CATESBEIANA* FROGLETS AND TADPOLES

A. FROGLETS

Animal:	Transition:	Length of Time Hours	Melanophore Index* at Fixation:
AF	white-to-black	21	4
DF	white-to-black	20	4-
EF	white-to-black	72	3++
FF	white-to-black	72	4
BF	black-to-white	18	2+
CF	black-to-white	22	2++
GF	black-to-white	72	2-
HF	black-to-white	72	2+
IF	black-to-white	17	2++
JF	black-to-white	17	2+

B. TADPOLES

Animal:	Transition:	Length of Time Hours	Melanophore Index at Fixation:
AT	white-to-black	24	3++
BT	white-to-black	24	4-

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* From Hogben and Slome, 1931. Fully light adapted animals have a melanophore index of 1, and dark adapted animals have indices of 4.5-5.0.

Animal:	Transition:	Length of Time Hours	Melanophore Index at Fixation:
CT	white-to-black	18	4-
IT	white-to-black	72	5-
JT	white-to-black	72	3++
DT	black-to-white	22	2+
ET	black-to-white	72	2-
FT	black-to-white	144	3-
GT	black-to-white	64	2+
HT	black-to-white	144	2++
KT	black-to-white	17	3-
LT	black-to-white	17	3++

TABLE II: A COMPARISON OF CYTOLOGICAL FINDINGS IN FROG PARS INTERMEDIA TYPE I CELLS UNDER VARIED BACKGROUND CONDITIONS.

Animal Type	Rough Endoplasmic Reticulum	Golgi Region	Intracisternal Droplets
<u>Rana catesbeiana</u>			
Light adapted	Flattened or slightly dilated	Relatively flattened with few granules	Absent
Dark adapted	Extensive whorls and expanded areas	Extensive, with many associated granules	Present
Light-to-dark Transitional	Some cells show expensions and whorls; many others nearby have flattened ER	Extensive in some cells, few granules in others	Present
Dark-to-light Transitional	Many cells show expansions and whorls; some have flattened ER	Extensive in many cells	Present
<u>Rana pipiens</u>			
Light adapted	Flattened or slightly dilated	Relatively flattened with few granules	Absent
Dark adapted	Extensive whorls and expanded areas	Extensive, with many associated granules	Rare or Absent

TABLE III: RELATIVE NUMBERS* OF CYTOPLASMIC GRANULES IN LIGHT AND DARK ADAPTED RANA PIPIENS ADULTS

A. LIGHT ADAPTED ANIMALS:

Animal	No. Cells	Percent Area of Granules:	Mean + Standard - Error
L67-6-1B	6	15.2%	
L67-6-9	9	14.1%	
L67-6-10A	1	30.6%	
L67-6-11	9	22.7%	
L67-6-12B	8	17.8%	
L67-6-14	1	18.3%	18.0% ± 1.2%

Total Cells = 34

B. DARK ADAPTED ANIMALS:

Animal	No. Cells	Percent Area of Granules:	Mean + Standard - Error
L-67-6-2	5	14.1%	
L-67-6-3	4	9.8%	
L-67-6-4	2	14.3%	
L-67-6-5	7	11.4%	
L-67-6-6	6	7.9%	
L-67-6-7	6	8.2%	
L-67-6-8	9	14.8%	12.1% ± 0.94%

Total Cells = 39

Using Student's t test, p lies between 0.02 and 0.05. Results appear to be statistically significant in comparing the means of Group A and Group B.

* Granules were measured according to the method of Loud (1962) whereby a sheet of lines one-half inch apart was placed over micrographs, and total lines covering the cells in question were measured in millimeters. The length of granules crossed by lines was also measured in millimeters,

and millimeters of granules per millimeters of total cytoplasm was expressed as a percent. Three counts were made per cell: (1) with lines horizontal (normal); (2) one-half inch below normal; and (3) 90° to normal. The mean for the three counts was used as a final count for each cell. All cells counted had visible nuclei. Results are presented as relative area occupied by cytoplasmic granules per estimated total area of cytoplasm for each cell studied.

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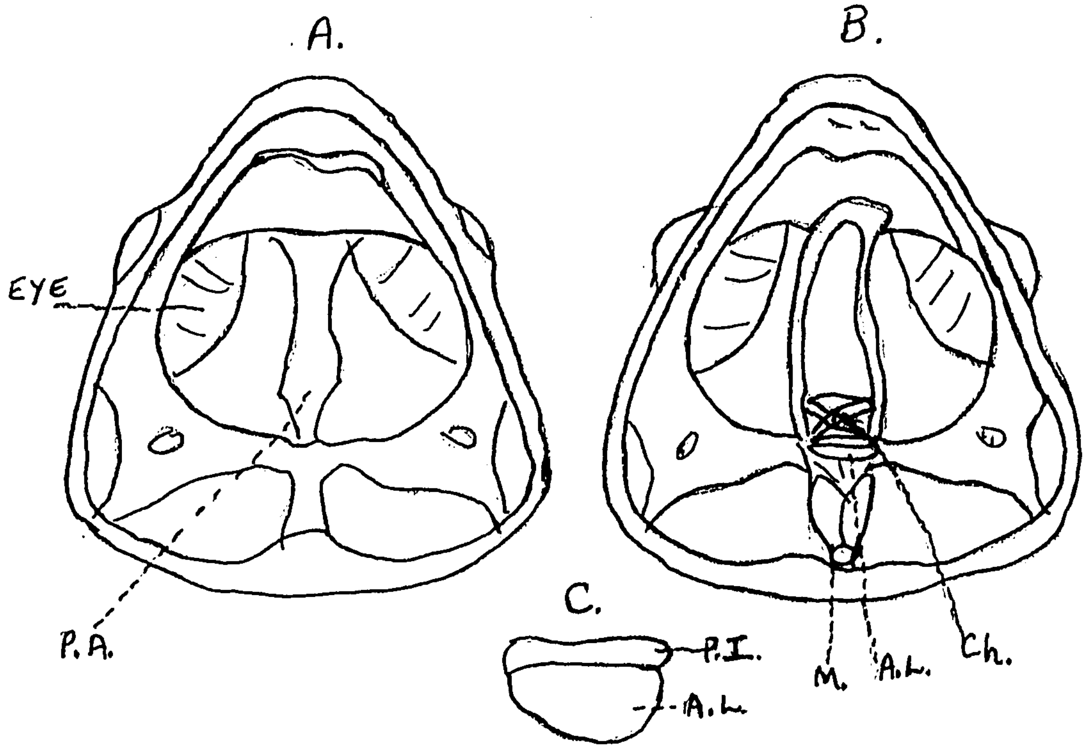
AUTOBIOGRAPHICAL STATEMENT

The author of this dissertation was born in New York City on October 24, 1942. After elementary and high school education in the public school system, she studied at The City College of New York, and received a Bachelor of Science degree in Biology in June, 1963. She was then accepted into the Graduate Faculties at Columbia University, and received a Master of Arts degree in Zoology in February, 1965. Upon receipt of the latter degree, the author applied to and was accepted into the Doctoral program in Biology, at The City University of New York. During the period at Columbia, she taught undergraduate embryology and comparative anatomy as a Teaching Assistant, and later, at City College, taught two semesters of General Biology. In the second year at the City University, she received a National Defense Education Act Title IV Fellowship, and has since pursued a course of study and research in preparation for the Ph.D. degree.

The author is the wife of Dr. Joel S. Saland, a member of the Pediatric house staff of Montefiore Hospital in the Bronx, New York, and is the mother of a son, Kenneth, born August 23, 1967.

FIGURE I: HYPOPHYSECTOMY IN FROGS



A. Head of a frog, ventral view
B. Ventral view, brain and hypophysis exposed
C. Hypophysis isolated

P.A. = parasphenoid bone ; Ch. = optic chiasma ;
M. = medulla oblongata ; A.L. = anterior lobe ;
P.I = pars intermedia

Modified slightly from V. Hamburger, 1960.

LIGHT MICROGRAPHS

FIGURE 2A: Light adapted bullfrog. Rounded or ovoid nuclei of predominant cells are observed in the pars intermedia (PI). Note columnar-like processes (C) adjacent to pars nervosa (PN). One micron. Toluidine blue epon. 0s04. X225.

FIGURE 2B: Dark adapted bullfrog. Dense droplets (arrows) are found in pars intermedia (PI) processes adjacent to the pars nervosa (PN). Pars anterior (PA). One micron. Toluidine blue epon. 0s04. X225.

FIGURE 2C: Light adapted <u>Rana pipiens</u>. Nuclei of pars intermedia (PI) appear similar to those of bullfrogs. Pars nervosa (PN). One micron. Toluidine blue epon. Glutaraldehyde-0s04. X225.

2 A

2 B

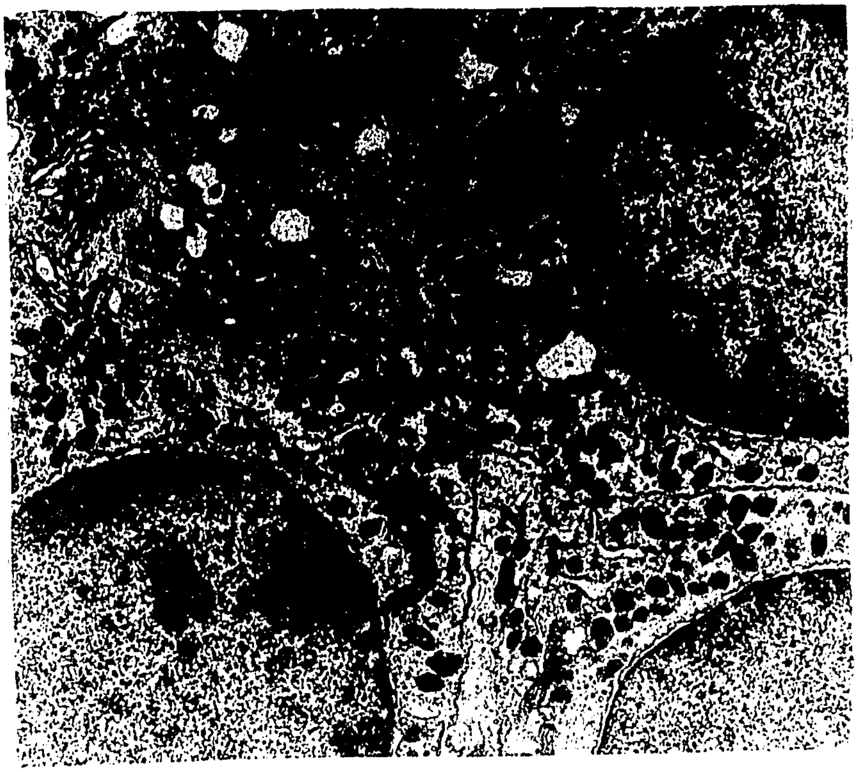
2 C

ELECTRON MICROGRAPHS

Light and Dark Adapted Bullfrogs

FIGURE 3: Light adapted bullfrog. Type I cell muclei (I) are prominent. A few dense granules are associates with the Golgi region (G). Nerve fibers containing small granules (N). Glutaraldehyde-0s04. X10,200.

FIGURE 4: Light adapted bullfrog. A Type II cell (II) sends processes amongst portions of Type I cells (I). Note varied densities and shapes of cytoplasmic granules, and small-granule nerve fibers (N). Glutaraldehyde-0s04. X9450.



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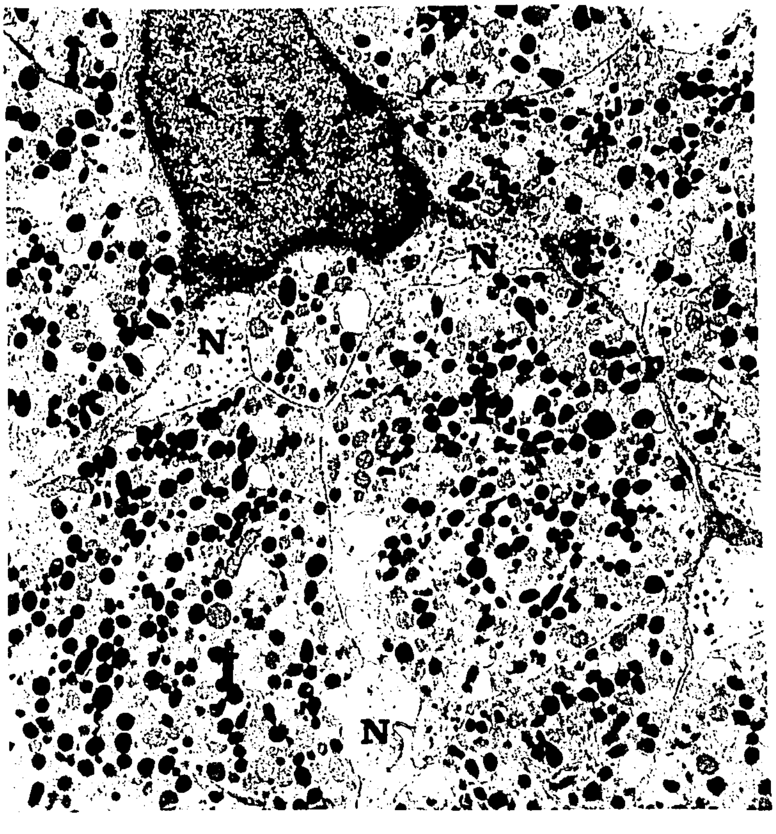

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FIGURE 5: Light adapted bullfrog. A fairly characteristic Golgi zone (G) of a light adapted animal is seen. Observe Type I cell nuclei (I). Arrows denote nuclear pores. Mitochondrion (M). Glutaraldehyde-OsO_4. X24,600.

FIGURE 6: Light adapted bullfrog. Two small-granule nerve fibers (N_1, N_2) are prominent. One (N_1) appears to be in synaptic contact (arrow) with a pars intermedia secretory cell. Rough endoplasmic reticulum (ER). Glutaraldehyde-OsO_4. X24,600.

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FIGURE 7: Light adapted bullfrog. A terminal (wide arrow) containing small, clear vesicles (SV) and a few dense core vesicles (slender arrow) is centrally located. Note that cytoplasmic granules are poorly preserved. Mitochondrion (M). OsO4. X 31,500.

FIGURE 8: Dark adapted bullfrog. A whorl of rough endoplasmic reticulum (ER) is in the midst of a Type I (I) cell. Arrows point to nerve fibers containing small granules. Glutaraldehyde-OsO4. X7200.

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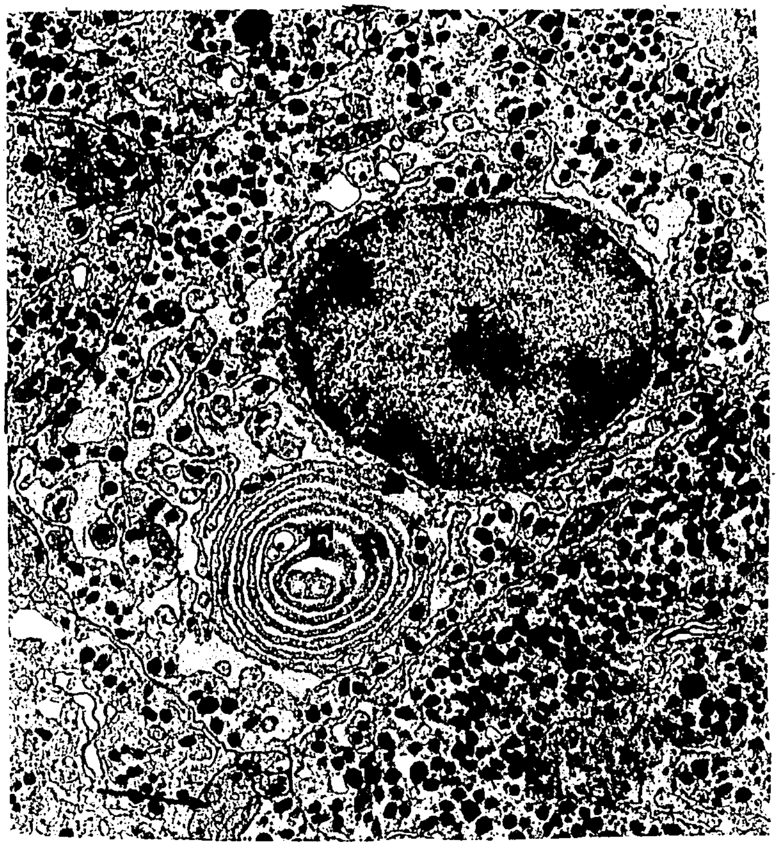

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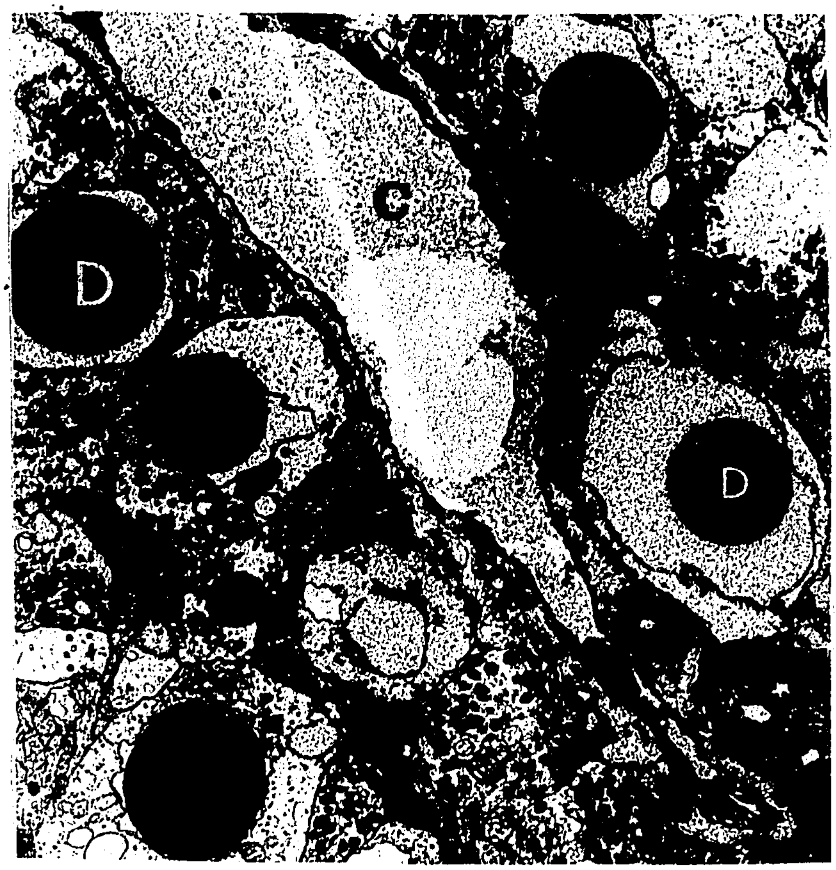
FIGURE 9: Dark adapted bullfrog. Area near neural lobe border. Dense droplets (D) lie in pars intermedia cell processes along a capillary (C). Droplets are bounded by membranes of rough endoplasmic reticulum (arrow). Glutaraldehyde-0s04. X8400.

FIGURE 10: Dark adapted bullfrog. Numerous dense granules are associated with the Golgi zone (G). Note dense and less dense cytoplasmic granules (arrows). Rough endoplasmic reticulum (ER). Glutaraldehyde-0s04. X 11,000.



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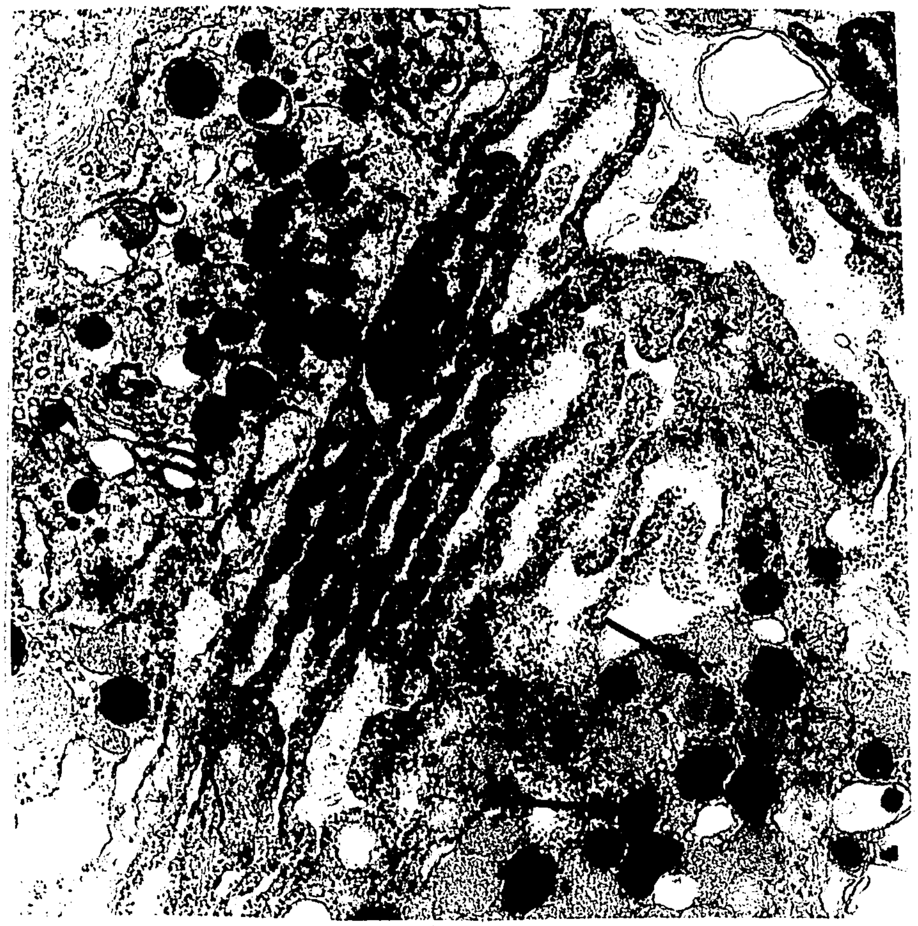

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FIGURE 11: Dark adapted bullfrog. Dense granules appear to be consensing in the Golgi region (G). Other cytoplasmic granules appear "caught" in intercisternal areas of expanded rough endoplasmic reticulum (ER). Arrow points to fine filamentous material within cisternae. Small-granule nerve fiber (N). Glutaraldehyde-0s04. X25,000.

FIGURE 12: Dark adapted bullfrog. Internal area of pars intermedia. Note small intracisternal droplet (D) and whorls of endoplasmic reticulum (ER). Small vesicles (arrows) may connect the ER with the Golgi zone (G). Multivesicular body (mvb). Glutaraldehyde-0s04. X20,400.

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FIGURE 13: Dark adapted bullfrog. A field similar to the type of Figure 12, but fixed in osmium alone. Note lack of membrane-bounded cytoplasmic granules. Endoplasmic reticulum (ER). Golgi zone (G). Lipid inclusion (L). OsO_4. X22,000.

FIGURE 14: Dark adapted bullfrog. Area of pars intermedia near the neural lobe border. Neurosecretory-like axons (NS) approach pars intermedia (pi) cell processes. Intracisternal droplets (D). Small-granule nerve fibers (N). Type II cell process (p). Glutaraldehyde-OsO_4. X23,800.

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FIGURE 15: Dark adapted bullfrog. Pars intermedia treated with acetone after glutaraldehyde fixation. Note breakup of membranes, presumably due to dissolution of lipids, but preservation of droplets (D) and smaller cytoplasmic granules. Type II cell (II) with process (p). Glutaraldehyde-OsO4. X7700.

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TRANSITIONAL BULLFROGS

FIGURE 16: Light-to-dark bullfrog, (Melanophore index 3.5). Cells appear much like those of light adapted glands. Type I cell nuclei (I). Golgi zone (G). Endoplasmic reticulum (ER). Small-granule nerve fibers (N). Glutaraldehyde-0s04. X16,500.

FIGURE 17: Light-to-dark bullfrog, (Melanophore index 3.5). Higher power micrograph of a group of cells from the same animal as in FIGURE 16. Note a few dense granules condensing in the Golgi region (G). Type I cell nuclei (I). Endoplasmic reticulum (ER). Glutaraldehyde-0s04. X21,000.

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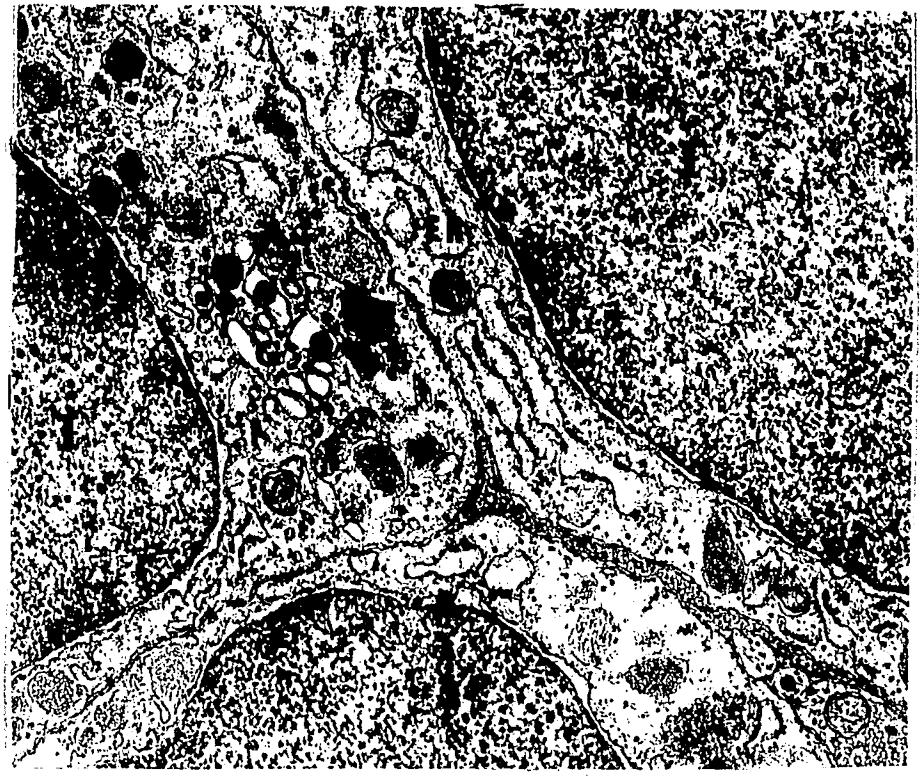

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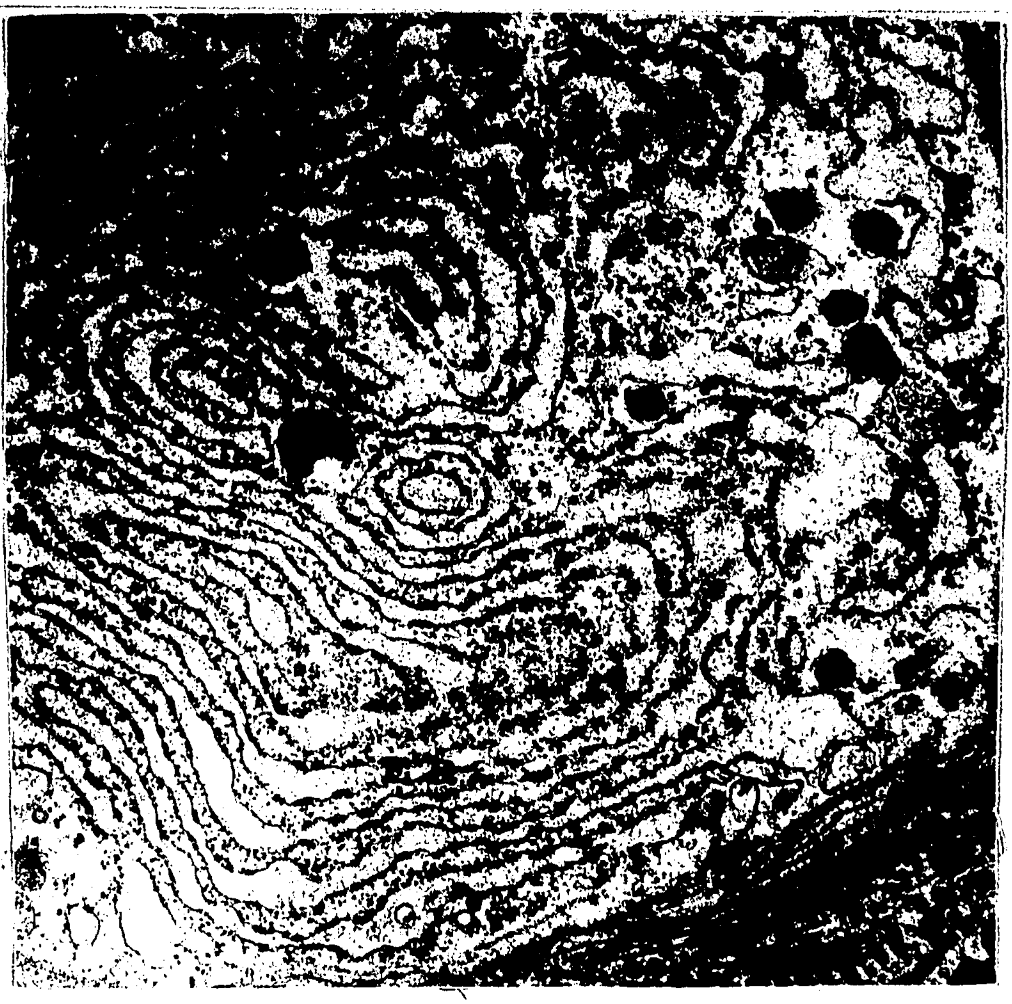
FIGURE 18: Light-to-dark bullfrog (Melanophore index 3.5). This whorl of endoplasmic reticulum (ER) was found among cells which showed flattened ER. Small-granule nerve fiber (N). Glutaraldehyde-OsO4. X21,000.

FIGURE 19: Light-to-dark bullfrog (Melanophore index 3.0). A small, dense intracisternal droplet (arrow) is found in a Type I cell process (I). Small-granule nerve fiber (N). Glutaraldehyde-OsO4. X25,100.



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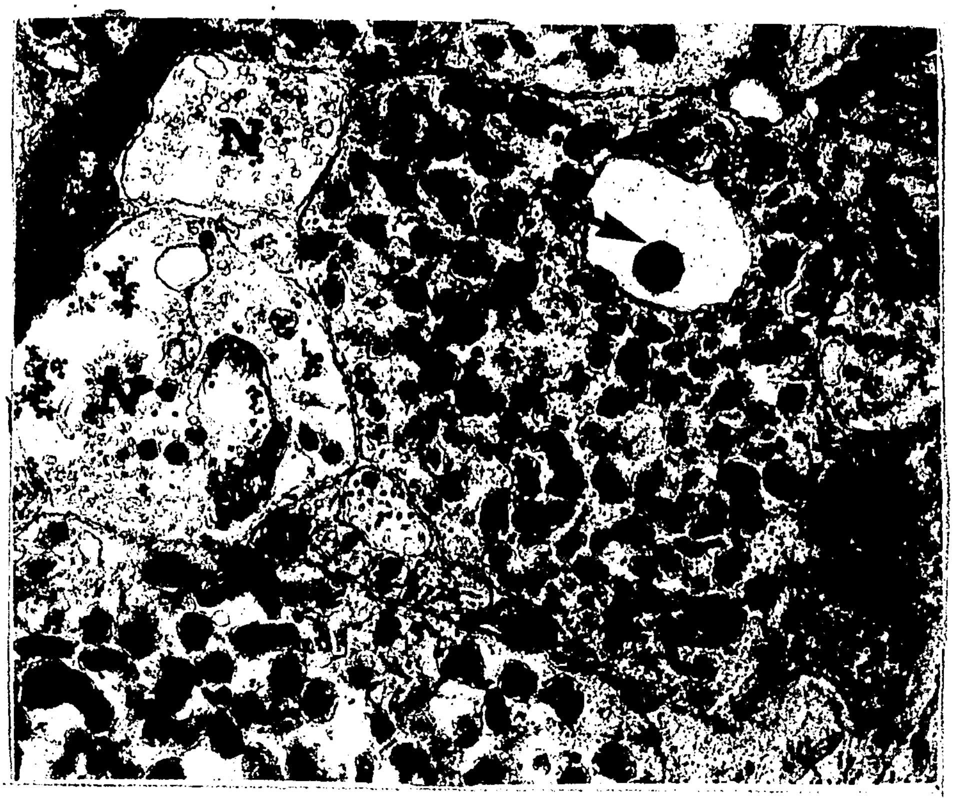

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FIGURE 20: Light-to-dark bullfrog (Melanophore index 3.5). There are a number of large, dense intracisternal droplets (D) in Type I cell processes near the neural lobe border. Arrow denotes rough endoplasmic reticulum surrounding filamentous material around a droplet. Glutaraldehyde-0s04. X21,000.

FIGURE 21: Light-to-dark bullfrog (Melanophore index 4.0). A nerve terminal is observed synapsing (arrow) upon a Type I cell (I). The terminal contains small, clear vesicles and dense core vesicles (one of the latter is encircled). Rough endoplasmic reticulum (ER). Free ribosomes (r). Note lack of cytoplasmic granules. 0s04. X31,500.

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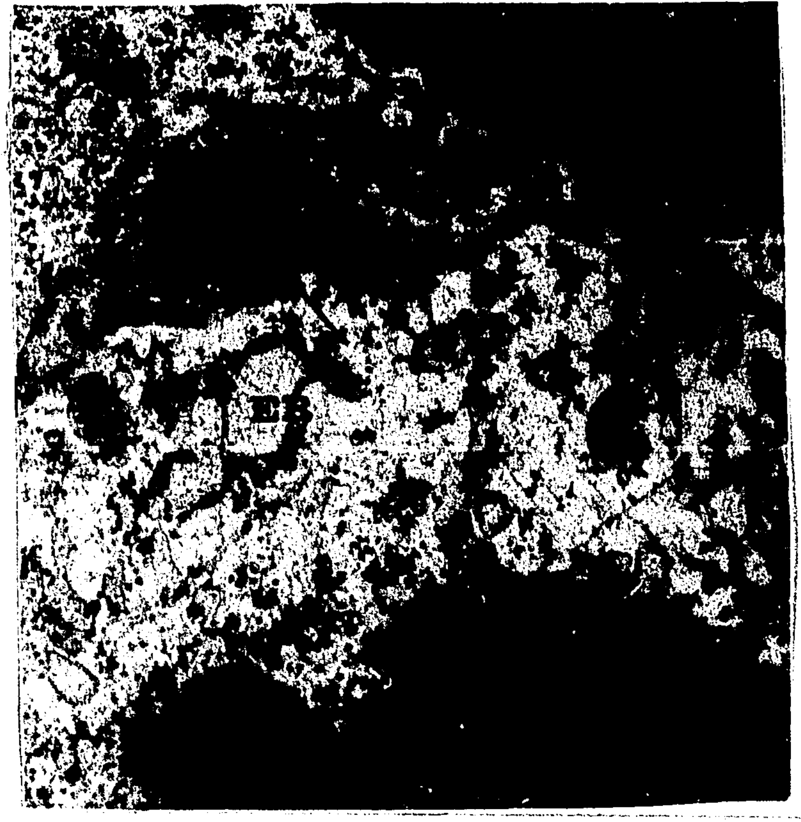

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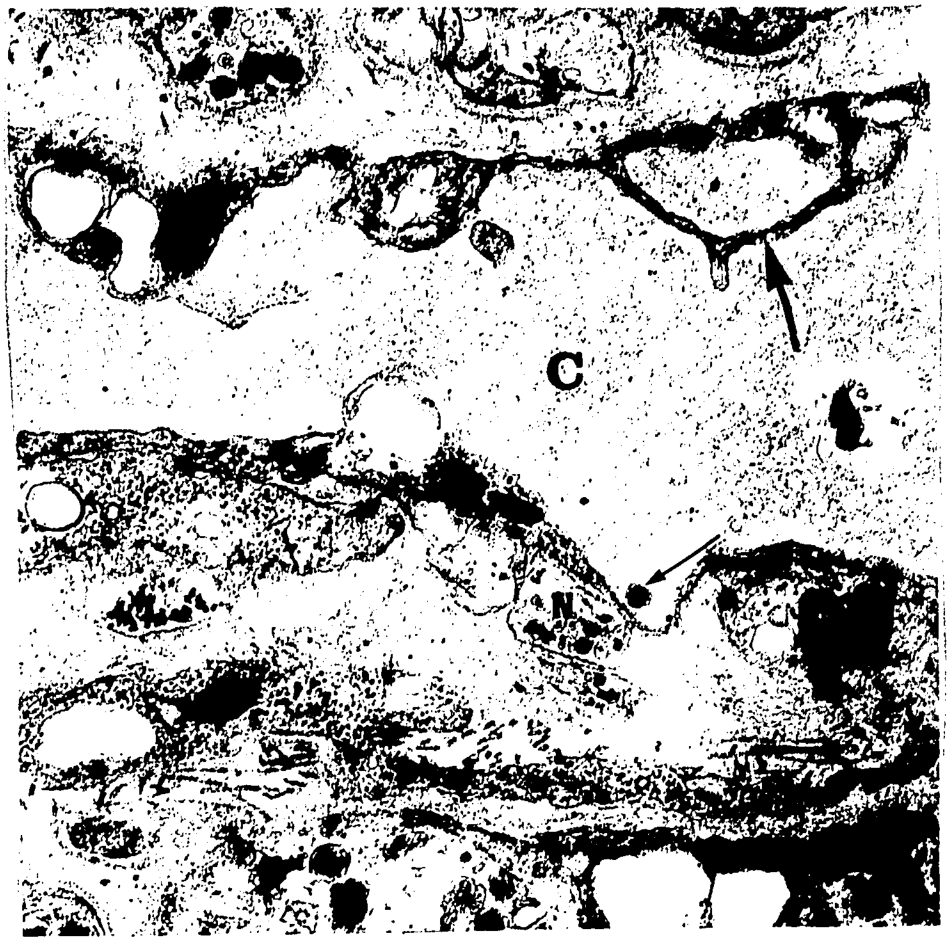
FIGURE 22: Light-to-dark bullfrog (Melanophore index 3.5). A small-granule nerve fiber (N) appears to terminate (arrow) on a Type I cell process (I). Rough endoplasmic reticulum (ER). Glutaraldehyde-OsO4. X31,500.

FIGURE 23: Light-to-dark bullfrog (Melanophore index 3.0). Pars intermedia Type I cells (I) bordering on a capillary (C) near the neural lobe. There appears to be a collagenous matrix (arrows) at the endothelial cell base. Very fine processes extend into the capillary lumen. Mitochondrion (M). Glutaraldehyde-OsO4. X25,100.

FIGURE 24: Light-to-dark bullfrog (Melanophore index 3.0). Another portion of the border area, same animal as in FIGURE 23. Note membrane-bounded granule (arrow) in the capillary lumen (artificially displaced?). Also note "blebbing" of endothelial cell cytoplasm on both sides of the lumen (wide arrow). Small-granule nerve fiber (N). Glutaraldehyde-OsO_4. X25,100.

FIGURE 25: Dark-to-light bullfrog (Melanophore index 2.5). Portions of four Type I cells (I). Note extensive Golgi zone (G) with dense granules, and partial expansion of endoplasmic reticulum (ER). Mitochondrion (M). Glutaraldehyde-OsO_4. X11,700.



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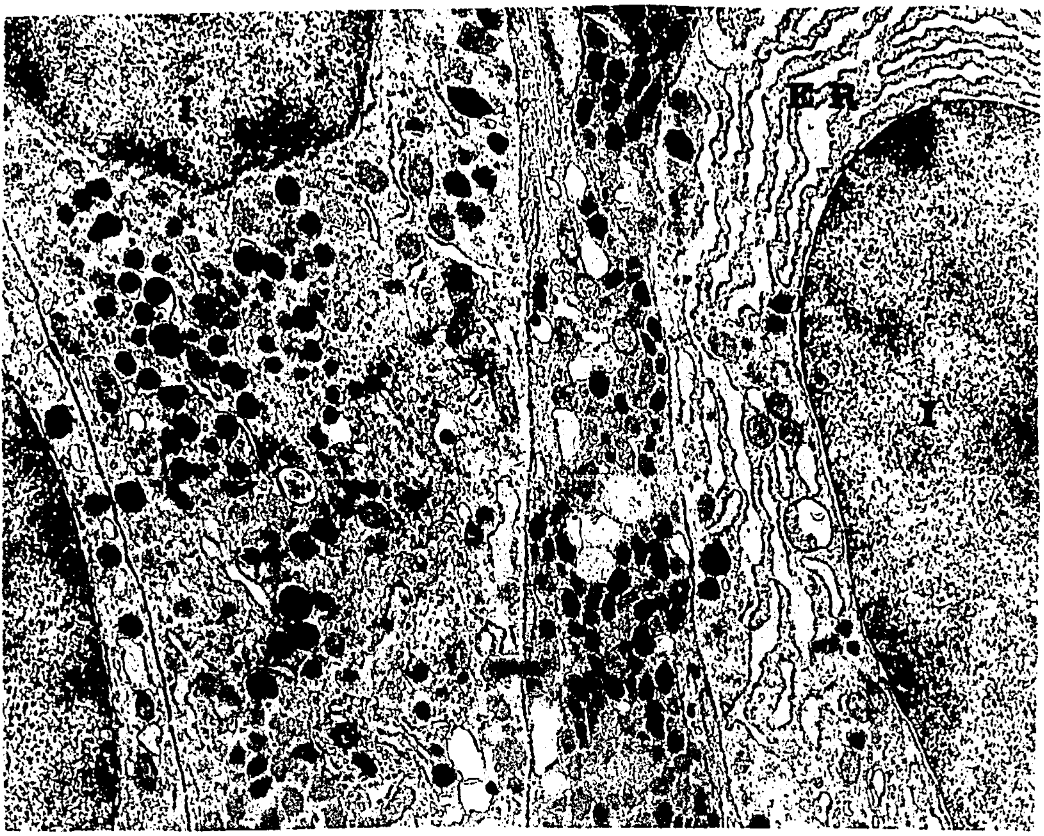

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FIGURE 26: Dark-to-light bullfrog (Melanophore index 2.5). There is whorl formation and expansion of rough endoplasmic reticulum (ER). Mitochondrion (M). Glutaraldehyde-OsO4. X23,800.

FIGURE 27: Dark-to-light bullfrog, (Melanophore index 3.0). The Type I cell (I) shows partial expansion of endoplasmic reticulum (ER), with a number of dense granules close to the Golgi zone (G). Type II cell (II). Glutaraldehyde-OsO4. X14,700.

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FIGURE 28: Dark-to-light bullfrog (Melanophore index 3.5). An extensive Golgi zone (G) is found in this cell. Note the presence of a ciliary rootlet (arrow) with clear striations. Mitochondrion (M). Glutaraldehyde-OsO4. X21,000.

FIGURE 29: Dark-to-light bullfrog (Melanophore index 2.5). Dense material can be observed condensing in a cistern of this Golgi zone (G). Multivesicular body (mvb). Glutaraldehyde-OsO4. X31,500.

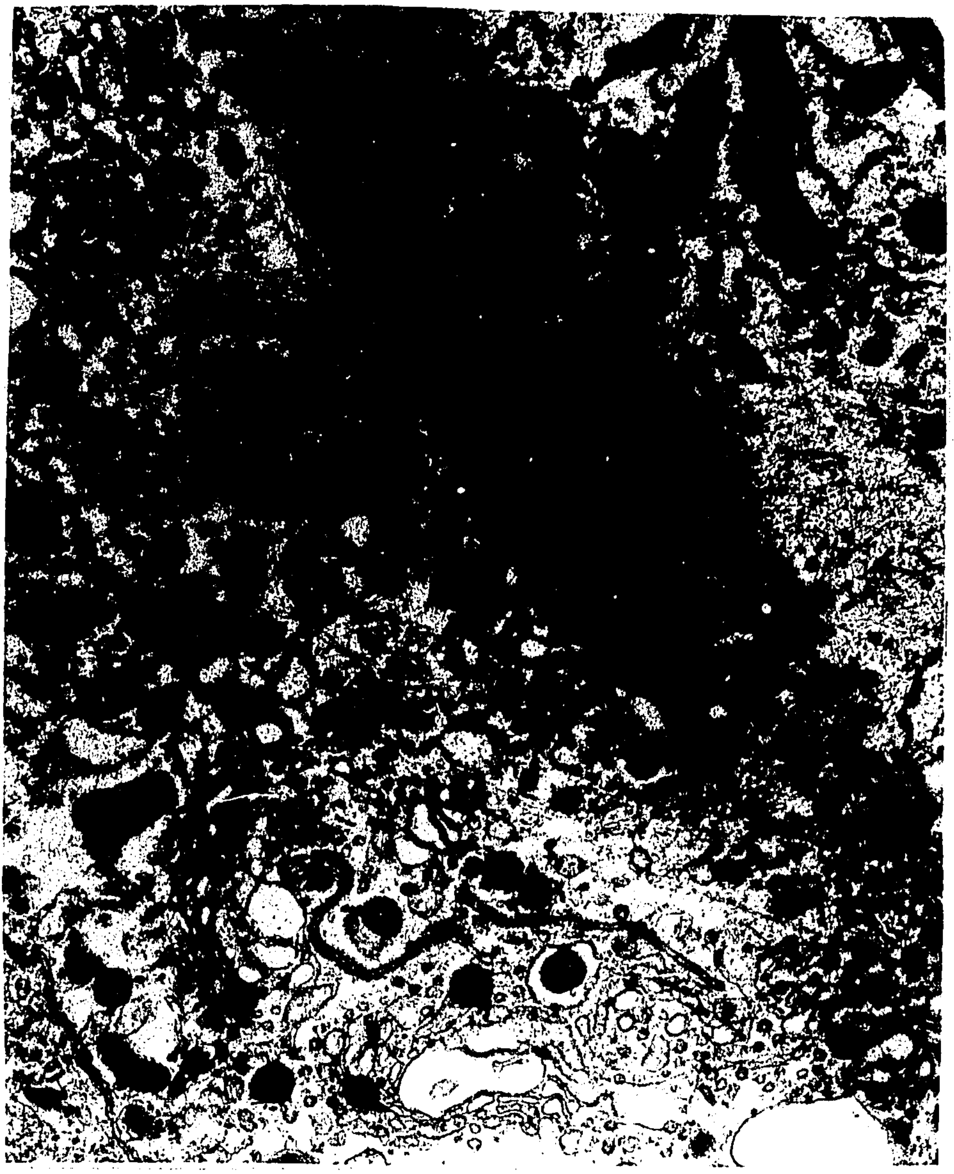

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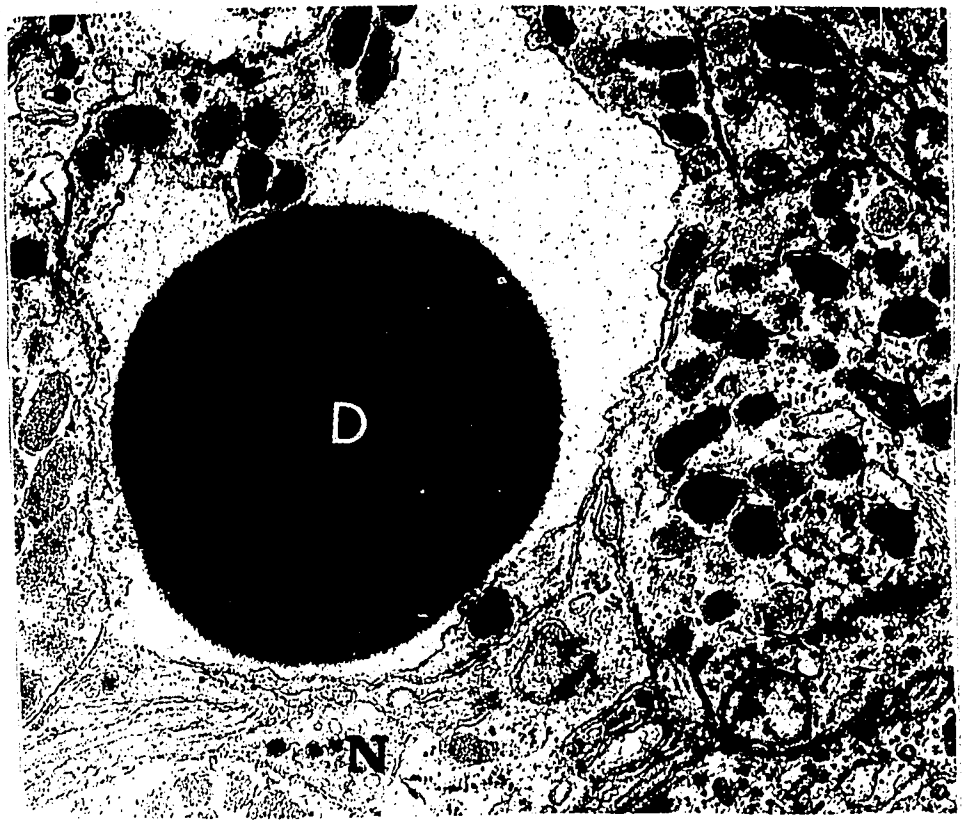
FIGURE 30: Dark-to-light bullfrog (Melanophore index 2.0). A large intracisternal droplet (D) is present in a process near the neural lobe. Small-granule nerve fiber (N). Glutaraldehyde-OsO_4. X21,000.

FIGURE 31: Dark-to-light bullfrog (Melanophore index 2.0). Same specimen as in FIGURE 30. A nerve fiber (N) is observed synapsing "en passant" (arrow) with a Type I cell process. Glutaraldehyde-OsO_4. X21,000.



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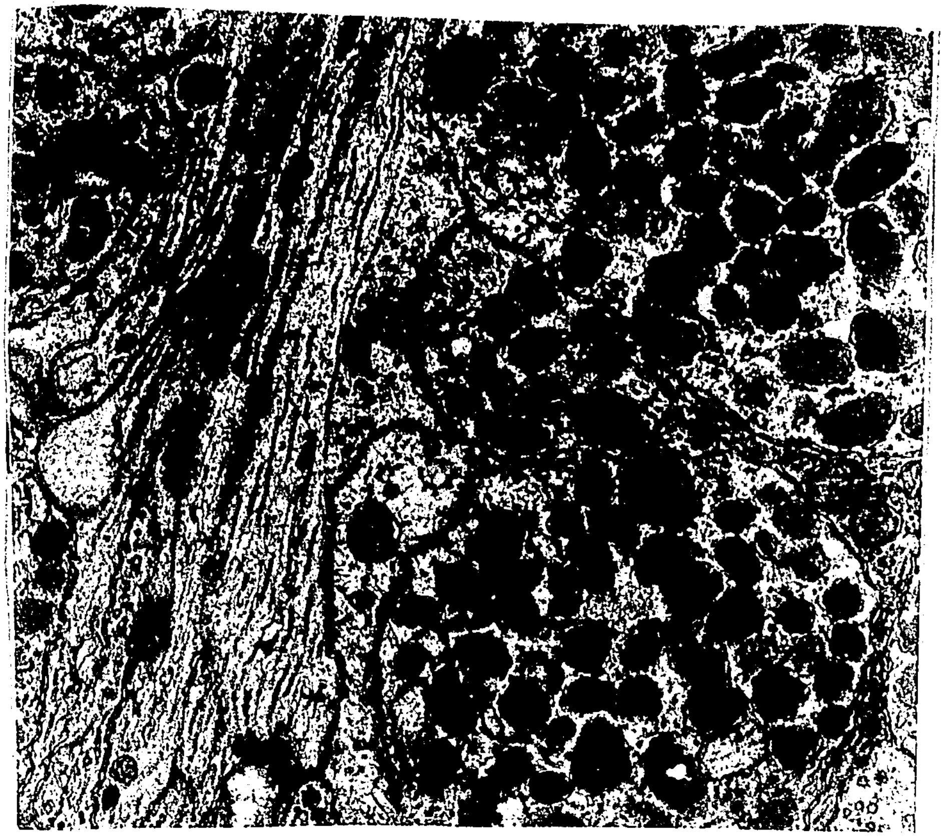

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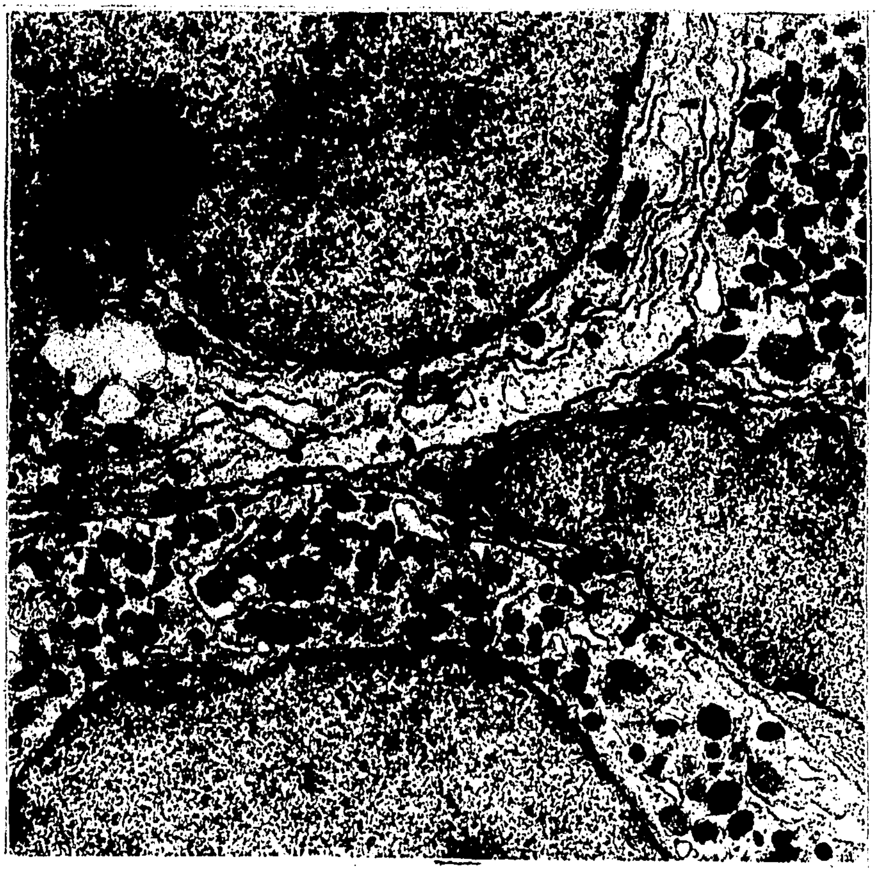
LIGHT AND DARK ADAPTED RANA PIPIENS

FIGURE 32: Light adapted Rana pipiens. Cells are very similar to those in bullfrogs. Note flattened rough endoplasmic reticulum (ER) and cytoplasmic granules. Type I cell nuclei (I). Type II cell nucleus (II) and process (p). Glutaraldehyde-OsO4. X10,100.

FIGURE 33: Light adapted Rana pipiens. The Type I cell (I) shows a somewhat more extensive Golgi zone (G) than is always observed in light adapted animals. The rough endoplasmic reticulum (ER) is flattened. Mitochondrion (M). Body which may be a lysosome (arrow). Glutaraldehyde-OsO4. X15,000.



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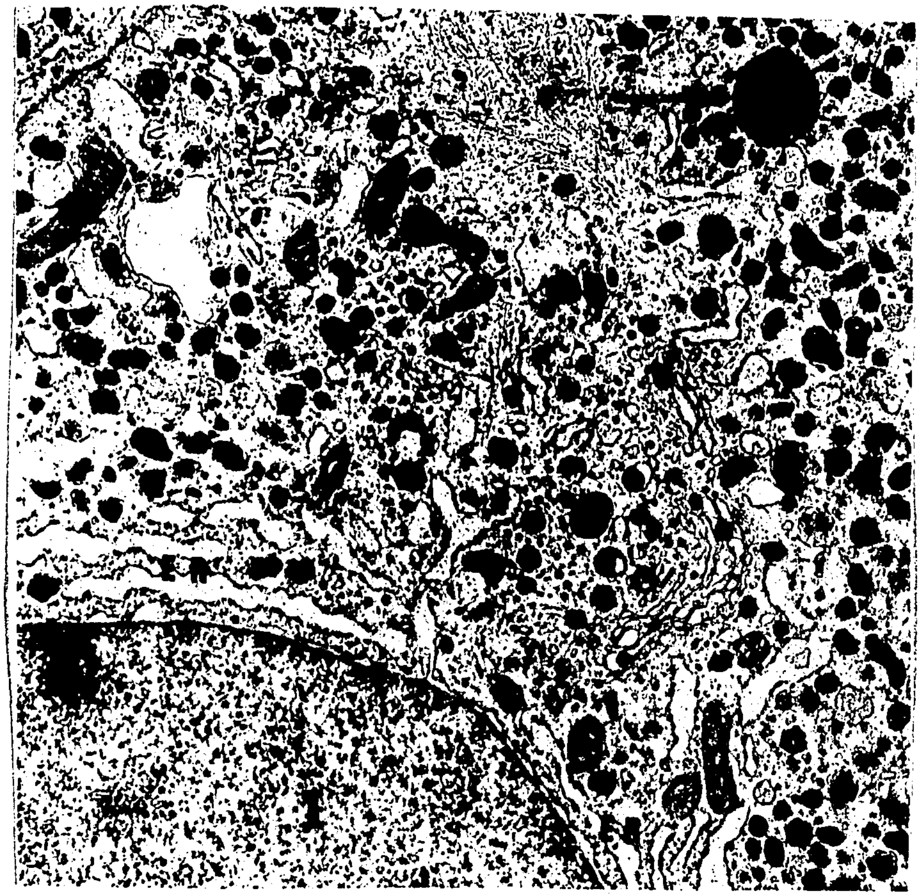

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FIGURE 34: Light adapted <u>Rana pipiens</u>. A small-granule nerve fiber (N) terminates (arrow) on a Type I cell (I). Sacs of rouch endoplasmic reticulum (ER). Glutaraldehyde-0s04. X15,700.

FIGURE 35: Light adapted <u>Rana pipiens</u>. Another nerve (N) terminal (arrow) which appears to be in synaptic contact with a Type I cell. Type II cell process (II). Glutaraldehyde-0s04. X24,100.

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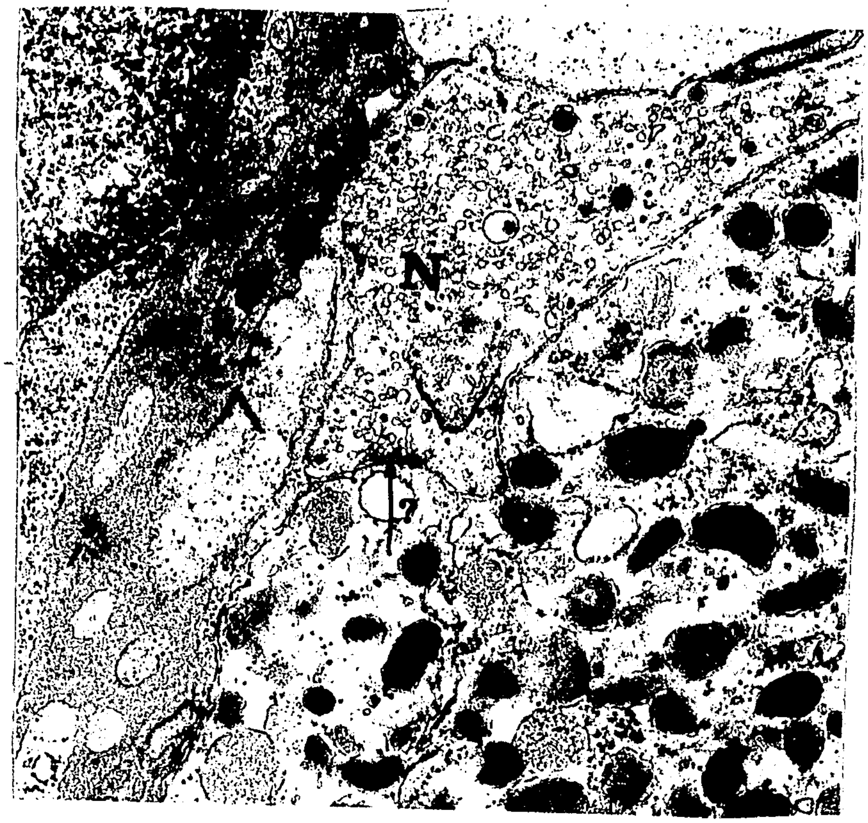

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FIGURE 36: Light adapted <u>Rana pipiens</u>. A "peptidergic" type nerve fiber (N; see Bargmann, et al, 1967) appears to terminate upon two pars intermedia cells. Note larger granules within the fiber which may be neurosecretory-type. Mitochondrion (M). Glutaraldehyde-OsO_4. X24,100.

FIGURE 37: Dark adapted <u>Rana pipiens</u>. Note similarities to bullfrog cells. There are stacks of expanded rough endoplasmic reticulum (ER) in Type I cells (I), and a fairly extensive Golgi region (G) with a number of dense cytoplasmic granules. Type II cell (II). Glutaraldehyde-OsO_4. X19,600.

FIGURE 38: Dark adapted <u>Rana pipiens</u>. Portions of two Type I cells are observed (arrow denotes cell membranes). The cell above shows extensive expansion of rough endoplasmic reticulum (ER), and a number of bodies which are probably lipids (L). Glutaraldehyde-OsO_4. X24,100.

FIGURE 39: Dark adapted <u>Rana pipiens</u>. There is a whorl formation of endoplasmic reticulum (ER), with fine filamentous material within cisternae (arrow). Type II cell process (p). Glutaraldehyde-OsO_4. X24,100.

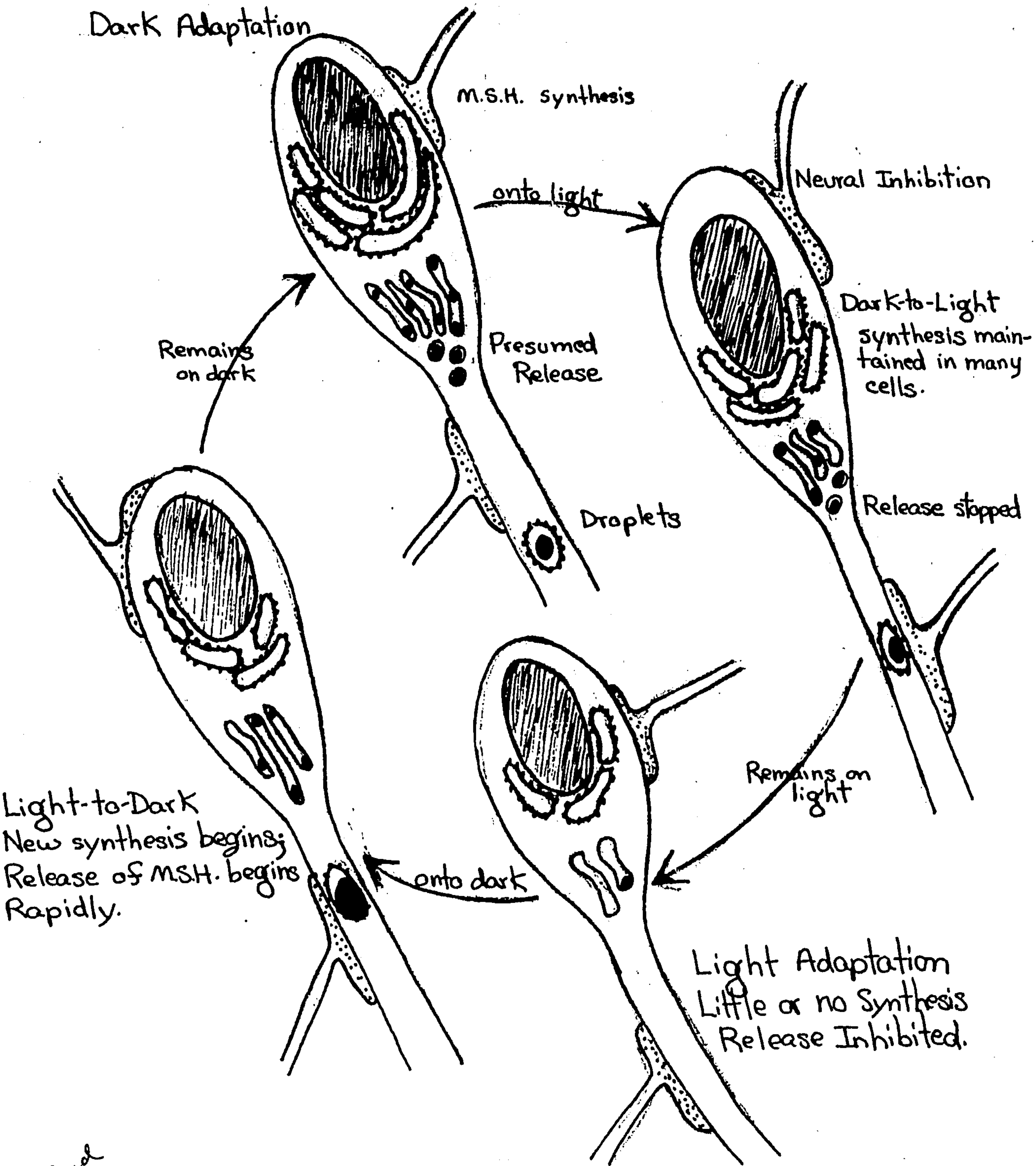
FIGURE 40: Dark adapted Rana pipiens. A small intracisternal droplet (D) was found in a Type I cell process. Nerve fiber (N) containing dense core vesicles. Glutaraldehyde-0s04. X24,100.

FIGURE 41: Dark adapted Rana pipiens. Another specimen, with two small intracisternal droplets (D). Droplets were only found in a few animals after searching a number of specimens. Nerve fiber (N). Portion of what may be a cilium (arrow with question mark). Glutaraldehyde-0s04. X19,300.

FIGURE 42: Dark adapted _Rana pipiens_. Observe the longitudinal section of a basal body portion of a cilium (arrow) and its rootlet (cr) in a Type I cell (I). A cluster of nerve fibers (N). Mitochondrion (M). Glutaraldehyde-OsO_4. X19,300.

FIGURE 43: Dark adapted _Rana pipiens_. Two small-granule nerve fibers (N) appear to be synapsing with a Type I cell (I). Rough endoplasmic reticulum (ER). Type II cell process (P). Mitochondrion (M). Glutaraldehyde-OsO_4. X18,000.

Figure 4.4: Hypothalamic Control of Frog Pars Intermedia with changes in Background Adaptation.



J. Saland