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THE ONTOGENY AND FUNCTIONAL SIGNIFICANCE OF LUTEINIZING HORMONE RELEASING HORMONE (LHRH) CONTAINING CENTERS OF THE BRAIN OF THE FRESHWATER TELEOST, XIPHOPHORUS MACULATUS

City University of New York PH.D. 1984

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by

LESLIE R. HALPERN-SEBOLD

A dissertation submitted to the Graduate Faculty in Biology in partial fulfillment of the requirements for the degree of Doctor of Philosophy , The City University of New York

1984

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

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The City University of New York

This dissertation is dedicated to the memory of my sister,
Nadine Andrea Halpern

and to the memory of my father,
Samuel David Halpern

whose love and encouragement live on in my son,
Seth Paul Sebold

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Abstract

THE ONTOGENY AND FUNCTIONAL SIGNIFICANCE OF LUTEINIZING HORMONE RELEASING HORMONE (LHRH) CONTAINING CENTERS OF THE BRAIN OF THE FRESHWATER TELEOST, *Xiphophorus maculatus*

by

Leslie R. Halpern - Sebold

Advisor: Martin P. Schreibman

The use of cytometric, immunocytochemical and radioimmunological methods has allowed the investigation of the ontogeny and functional significance of LHRH containing centers in sibling platyfish (*Xiphophorus maculatus*) genetically determined to reach sexual maturity at different ages and in adult fish which were hypophysectomized and given replacement therapy with gonadotropin.

There is a sequential development of three immunoreactive (ir-) LHRH centers in the brain that is directly related to stage, not age of sexual maturation. The first region to contain ir-LHRH is the nucleus olfactoretinalis (NOR). Ir-LHRH then appears in the nucleus preopticus periventricularis (NPP), followed by the nucleus lateralis tuberis (NLT). This anterior to posterior sequence has been termed as the "cascade effect" and is essentially similar in both early and late maturing genotypes, except that in late maturers, specific steps of the "cascade effect" take place at similar developmental stages, but in older animals, and require more time to be completed.

The appearance of these LHRH producing centers in the brain precede and are presumably essential for the completion of gonadotrop development and the subsequent maturation of the gonads. A direct correlation is found between the number of ir-LHRH containing perikarya in the brain and number of ir-GTH cells in the pituitary of both early and late maturers.

In both genotypes, the number of ir-cells are similar in the NOR and PI, but in late maturers, the number of ir-cells in the NPP,NLT and CPD are significantly less.

The dynamics of the interaction between LHRH and GTH are further illustrated by the effect of hypophysectomy and hormone replacement. ICC and RIA results demonstrate that hypophysial removal produces distinct changes in the distribution and quantity of LHRH in the brain. A decrease in the total LHRH content occurs in the brains of hypophysectomized animals, which is partially restored by the administration of gonadotropin.

The different responses of these ir-LHRH containing centers to hypophysectomy and the sequential accumulation of ir-LHRH in the NOR,NPP and NLT between birth and puberty suggest that they differ in their roles in regulating BPG axis function.

The results of this study also indicate that the site(s) and mechanism(s) of the P gene are highly complex phenomena and that the P gene may express itself, either directly or indirectly , at various levels of the nervous and endocrine systems.

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This thesis project could never have been undertaken without the support and encouragement given to me by my mentor and friend, Dr. Martin P. Schreibman. I would like to express my appreciation to Martin for introducing me to the exciting world of fish physiology, supporting me through all the happy and sad times I encountered during my graduate training and most of all, teaching me what the word committment means. I hope to repay him by doing the same for others in the future.

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contribution to the field of neuroendocrinology.

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Introduction:

For many years, considerable effort has been spent seeking understanding of the mechanism(s) that control sexual maturation. Puberty in vertebrates, has its basis in developmental events within the brain-pituitary-gonadal (BPG) axis. Luteinizing hormone releasing hormone (LHRH), also known as gonadotropin releasing hormone (GnRH), regulates the synthesis and release of gonadotropins from the anterior pituitary gland of mammals (Schally et. al., 1971; Ojeda et. al., 1980, 1983; Reiter and Grumbach, 1982). During gestation a relationship is established between the appearance of LHRH in specific regions of the brain and the functioning of the hypophysis (guinea pig, Barry and DuBois, 1974; Silverman and Zimmerman, 1978; Schwanzel-Fukuda and Silverman, 1980; rat, Setalo et. al., 1978; Daikoku et. al., 1980; mouse, Hoffman et. al., 1978; Gross and Baker, 1979; humans, Pugnon et. al., 1977; Barry, 1979; monkies, Silverman et. al., 1977; Paulin et. al., 1977).

Considerably less is known about the interrelationships of the brain, pituitary and gonads in non-mammalian vertebrates. In teleosts, as in mammals, the brain and pituitary are anatomically and functionally linked (Peter, 1970, 1973, 1982, 1983a,b), however, in fish but not mammals, axons of neurosecretory cells end on or near gonadotropic cells (Schreibman et. al., 1973, 1979; Kaul and Vollrath, 1974; Peter, 1983a, b; Nagahama and Peter, 1982; Zambrano, 1970, 1971; Peute et. al., 1976; Ball, 1981). Two types of fibers, A and B, originating in the nucleus preopticus (NPO) and nucleus lateralis tuberis (NLT) of the hypothalamus respectively, innervate the gonadotropic cells (Peter and Nagahama, 1976; Ball, 1981; Peter, 1983a,b). Electrical lesioning of axons emanating from perikarya of the NLT, results in a decrease in the levels of

circulating gonadotropin and gonadosomatic indices (Peter, 1982, 1983; Peter et. al., 1978; Peter and Crim, 1979; Peter and Paulencu, 1980).

The existence of LHRH in teleosts has been established and its role appears to be similar to the one it serves in mammals. Analogues of mammalian LHRH can induce gonadotrop proliferation and subsequent gametogenesis in sterile hybrids of platyfish (Bao and Kallman, 1982), goldfish (Peter, 1982; Peter, 1983) and medakas (Chan, 1977). Similarly, crude hypothalamic extracts have been isolated from the teleost brain that elicit LHRH-like activity both in vivo and in vitro and a gonadotropin releasing hormone has been identified in several teleosts using biochemical methods (Cyprinus carpio, Breton et. al., 1975; Carassius auratus, Crim et. al., 1976; Pseudopleuronectes americanus, Crim et. al., 1981; Sarotherodon mossambicus, King and Millar, 1980; Barnett et. al., 1979, 1982; Sherwood et. al., 1983). The LHRH molecule of teleosts contains regions of amino acid sequences that are similar to mammals, thus indicating conservation of structure throughout vertebrate evolution (King and Millar, 1980; Barnett et. al., 1982; Sherwood et. al., 1983).

Immunocytochemistry has been useful in identifying centers containing immunoreactive (ir-) LHRH and their neuronal paths to the pituitary. Ir-material has been identified in perikarya and axons of the nucleus olfactoretinalis (NOR), nucleus preopticus periventricularis (NPP) and nucleus lateralis tuberis pars posterioris (NLTp) in the brain, and in axonal endings supplying cells containing ir-gonadotropin (GTH) in the caudal pars distalis of the pituitary and in cells of the pars intermedia (PI) in several teleosts platyfish (Schreibman et. al., 1979; Munz et. al., 1981, 1982); goldfish (Kah et. al., 1984); rainbow trout (Dubois et. al, 1979; Goos and Muralhanaglu, 1977); three-spine

stickleback (Borg et. al., 1982) and carp (Nozaki and Kobayashi, 1979; Nagahama and Peter, 1982). There have been no studies on the ontogeny of LHRH-containing centers in the brain of teleosts and its functional significance in the development of sexual maturation.

Xiphophorus maculatus (the platyfish) can be used as a model system for investigating the interaction of the genome with the neuroendocrine system in determining the timing of puberty (Kallman and Schreibman, 1973; Kallman et. al., 1973; Schreibman et. al., 1973, 1979, 1982a, b, c, d; Schreibman and Kallman, 1977, 1978). The age of onset of sexual maturation depends upon at least five alleles at the P locus. These alleles are located on the sex chromosome(s) and are linked to a number of pigment genes (Kallman and Schreibman, 1973; Kallman et. al., 1973; Kallman and Borkoski, 1978). Each combination of P alleles (P^1 to P^5) determines the age and size at which the gonadotrops of the pituitary proliferate and become physiologically active and sexual development ensues (Kallman and Schreibman, 1973; Kallman and Borkoski, 1978; Schreibman and Kallman, 1978; Bao and Kallman, 1982). Specific genetic crosses can be generated to produce siblings that reach puberty at different chronological ages (Kallman and Schreibman, 1973; Schreibman and Kallman, 1977; Kallman and Borkoski, 1978). Changes in the brain-pituitary-gonadal (BPG) axis therefore occur in clear definite stages that are easily identified and thus permit the investigation of puberty phenomena in different genotypes (Schreibman et. al., 1982a, b, c).

Pituitaries from late maturing animals do not possess a functional gonadotropic zone at the same chronological time as early maturing sibs (Kallman and Schreibman, 1973; Schreibman and Kallman, 1977, 1978; Kallman and Borkoski, 1978). Since pituitary development differs between genotypes and since LHRH is needed for a proliferation of gonadotrop

activity, a study was undertaken to examine the ontogenic interaction between the appearance of LHRH-containing centers in the brain and the development of pituitary-gonadal activity in early and late maturing platyfish. This study is divided into two parts:

(1) The ontogeny of luteinizing hormone releasing hormone (LHRH) centers in the brain of platyfish genetically produced to reach puberty at two different ages in order to correlate the development of hormone-containing brain regions with pituitary-gonadal structure and function.

(2) Hypophysectomy and replacement with fish gonadotropin in sexually mature platyfish, to examine the dynamics of the interaction of LHRH and GTH synthesis and secretion.

Materials and Methods

The platyfish, Xiphophorus maculatus, is a freshwater poeciliid whose habitat ranges from southeastern Mexico to Belize. The original stocks were collected for the New York Zoological Society in 1939 by Myron Gordon. Descendents of these stocks and those more recently collected are maintained and bred at the Osborn Laboratory of Marine Sciences of the New York Aquarium. Some stocks collected in the Rio Jamapa (strain 163A and 163B) have been inbred for over 60 generations.

The sex-linked gene, P, determines the age at which the onset of sexual maturity occurs in male and female platyfish (Kallman and Schreibman, 1973; Kallman et al., 1973; Schreibman and Kallman, 1977; Schreibman and Kallman, 1978). Five P alleles (P^1 to P^5) have been identified in natural populations and laboratory stocks (Kallman and Borkoski, 1978). These alleles determine the time at which the brain-pituitary-gonadal axis becomes active and sexual maturation ensues. The P factors are carried on both sex chromosomes, X and Y, and are closely linked to a number of color genes that serve as phenotypic markers for the P locus (see table 1).

The 193 male and female platyfish used in these studies were derived from stocks of the Genetics Laboratory of the Osborn Laboratories and their descendents that have been maintained and inbred at Brooklyn College. Siblings, or fish of the same age and genetic strain, were used in each experiment (see table 2) for genetic crosses. All animals were fed three times daily a diet of liver-cereal mixture, brine shrimp nauplii or dried shredded shrimp and/or commercial flake food (Tetramin or equivalent).

All fish were kept under a 16 hour light - 8 hour dark cycle, at appoximately 23°C in aquaria containing "aged" New York City tap water, gravel and plants.

Anal Fin Metamorphosis

The transformation of the anal fin of males into a gonopodium, which transmits sperm during copulation can serve as an *in vivo* indicator of the stage of sexual maturation and of sex steroid levels (Grobstein, 1948; Schreibman *et al.*, 1982). There are six clearly defined stages in this anal fin metamorphosis (see Table 3) which are dependent upon increasing levels of androgens and can thus be used to indicate the stage of sexual maturation.

The various stages of gonopodial development are described below and are correlated with the developmental phase of the BPG axis (see Table 4);

(1) Unmodified Anal Fin: This is characteristic of males from birth to stage 1. Pituitary glands contain all cell types except for the gonadotropic zone of the ventral caudal pars distalis (vCPD) (Schreibman, 1964). Testes are comprised of two lobes, with spermatogonia on their periphery, surrounding a central connective tissue region (Schreibman *et al.*, 1981). Ovaries are composed of previtellogenic oogonia (see Schreibman *et al.*, 1982, for details).

(2) Gonopodial Stage 1: The 3rd, 4th and 5th rays of the anal fin are elongated. It occurs at four to five weeks of age in all males, regardless of genotype. The gonadotropic zone of the vCPD contains only a few chromophobic cells. Spermatogenesis proceeds up to the spermatocyte stage and oogenesis proceeds up to the yolk deposition stage (Schreibman and Kallman, 1977, 1978).

(3) Gonopodial Stage 2: The distal ends of the fin rays continue to elongate and thicken to form the forerunner of the "holdfast" structure. This occurs as early as 10-11 weeks in $\underline{P}^1\underline{p}^2$ animals and 25-26 weeks in $\underline{P}^2\underline{p}^5$ fish. Gonadotrops in the vCPD increase in number and display signs of secretory activity. Secondary spermatocytes are now prevalent and the

beginning of spermiogenesis is in evidence. Oocytes begin to accumulate yolk granules (Schreibman and Kallman, 1977).

(4) Gonopodial Stage 6: The gonopodium appears as an elaborate bony structure. This occurs at 18-20 weeks in $\underline{P}^1\underline{P}^2$ animals and 59-73 weeks in $\underline{P}^2\underline{P}^5$ animals. The gonadotropic zone and gonads are completely developed at stage 6 and are indistinguishable in early and late maturing males.

In females sexual maturity is determined by the presence of at least one yolky oocyte and is ascertained at autopsy.

Hypophysectomy (H):

a) Temporal Study

Thirty, 34 week-old, sexually mature female platyfish of the Jamapa stock (JP 163A, generations 57, 58 and 59) were used.

One week before hypophysectomy, fish were transferred from freshwater into tanks containing aerated 1/3 sea water, gravel and no plants. Fish were anestetized in 0.04% tricone methane sulfonate (MS-222), and the pituitary was removed according to the method of Schreibman and Kallman (1966). Sham-operated and hypophysectomized animals were returned to 1/3 sea water. At 1, 3 and 5 weeks following surgery, 7 H and 3 sham-operated animals were sacrificed and processed for ICC analysis.

Hypophysectomy and Gonadotropin (GTH) Administration:

Sixty-five, 34 week-old, sexually mature female platyfish of the Jamapa stock (JP 163A, generations 57, 58 and 59) were used in this study.

Fish were operated on as indicated above and on alternate mornings beginning on the fifth week following surgery all animals were injected intraperitoneally, with either 5 ul of 0.6% NaCl or 10 ug salmon GTH (SG-G100) in 5 ul of 0.6% NaCl, per gram body weight. A total of 5 injections were administered. Animals were decapitated and processed for analysis 2 hours after the fifth injection (6 weeks post-op).

The three experimental groups were:

Group 1: Sham-operated-saline injected; of the 15 animals, 8 were prepared for radioimmunoassay RIA and 7 were processed for ICC study.

Group 2: Hypophysectomized-saline injected; of the 25 animals, 12 were prepared for RIA and 13 processed for ICC.

Group 3: Hypophysectomized-GTH injected; of the 25 animals, 14 were prepared for ICC and 11 for RIA.

Histology:

Animals were decapitated just posterior to the operculum and heads and bodies were placed in Bouin's fluid containing acetic acid for twenty-four hours, under vacuum (23 mm Hg) at room temperature. Tissues were decalcified (Decal, Omega Co.) dehydrated (Zirkle normal butyl alcohol series, Krajan, 1940), and subsequently embedded in "Paraplast" (Monoject) under a dissecting microscope. Five micra (um) thick serial sections were cut in the transverse or sagittal plane, mounted on gelatin coated slides and processed for immunocytochemical and/or histological evaluation or confirmation of hypophysectomy.

The following histological stains were utilized:

(1) Nissl Stain: to demonstrate basophilia in neurons processed for histological and immunocytochemical evaluation.

(2) Masson's Trichrome: to analyze pituitary cell types and neurons and as a counterstain for immunocytochemically treated slides.

(3) Harris' Hematoxylin and Eosin: to evaluate the stages of gametogenesis.

Immunocytochemistry (ICC):

The indirect immunoperoxidase anti-peroxidase (PAP) method of Sternberger et al., (1970) was employed for all immunocytochemical

evaluations using rabbit antiserum to synthetic LHRH (anti-LHRH; Goos et al., 1976) or to the beta-subunit of purified carp gonadotropin (anti-cGTHB; Burzawa-Gerard et al., 1976; Fontaine and Burzawa-Gerard, 1978). For methods of preparation of antibodies and their conformation of specificity, see discussion of methods..

Anti-LHRH was used at a concentration of 1:500 or 1:1000, anti-cGTHB at dilutions ranging from 1:1200 to 1:3000, and rabbit peroxidase anti-peroxidase (PAP) complex (Cappell) at a dilution of 1:400. All dilutions were made using sodium phosphate buffered saline (PBS 0.01M; pH 7.6). In order to verify the specificity of the antisera, control procedures, in which adjacent sections were alternatively incubated with anti-LHRH or anti-GTHB, or any one of the following, were employed:

(1) Normal sheep serum and/or Normal rabbit serum in the same dilution as the primary antisera.

(2) Phosphate buffered saline.

(3) Primary antiserum which had been preincubated (24 hrs. at $4^{\circ}C$) with corresponding antigen (synthetic LHRH or carp GTH) or heterologous antigen rat neurophysin protein or thyroglobulin (Sigma) at a concentration of 1-10 ug per 1 ug antibody, in final dilution.

(4) Other LHRH antisera prepared against varying specific portions of the amino acid sequence of LHRH:

(a) Arimura 710: 1:250-1:1000 (prepared against the N-terminus)

(b) Arimura 743: 1:250-1:1000 (prepared against the C-terminus)

(5) Antisera prepared against other pituitary glycoprotein hormones or their subunits:

(a) Anti-cGTH (alpha subunit); 1:1000-1:3000.

(b) Antiserum to the beta subunit or entire molecule of human thyroid stimulating hormone (TSH); 1:1000-1:3000.

Radioimmunoassay:

Animals were decapitated and brains (with or without attached pituitaries) were dissected out of the cranium. Individual brains were immediately placed into 500ul of cold 1N acetic acid, homogenized and centrifuged at 2900 RPM at 4°C for 30 minutes. The supernatant was frozen immediately and stored at -56°C until ready for analysis. The pituitaries of fish in the same control group were pooled but otherwise processed in the same way.

The RIA was performed according to the method of Nett et al., (1973), as modified by Araki et al., (1975). The LHRH antiserum, specific batch number 42, prepared by Niswender against the C-terminus of the decapeptide shows no cross-reactivity with a number of other neuropeptides and various LHRH analogues. Twenty-five, fifty, seventy-five, and one hundred ul aliquots were assayed to arrive at a dilution curve (Plate 1). The antiserum were used at a dilution of 1:50,000. The sensitivity of the assay was 5 pg/ml and results were calculated as pg/ml extract examined. The parallelism observed between the LHRH reference standard and the fish brain extracts (Plate 1) justifies the application of this RIA procedure to platyfish. Plate 2 is the actual curve used to evaluate the effect of hypophysectomy on ir-LHRH levels.

Morphometric Analyses:

(1) Volume Measurements of Brain Nuclei; NOR, NPP and NLT: The method described below is modification of the one developed by Schwanzel-Fukuda and associates (1981). The boundaries of the NOR, NPP, and NLT, as observed from Masson and Nissl stained material, were drawn onto graph paper, composed of 1mm squares using a camera lucida (Leitz) at a magnification of 950X. Drawings were made at intervals of 10 microns (every other sagittal section in both hemispheres of each brain nucleus).

The volume of each brain nucleus was then obtained by taking the average volume per section and multiplying it by the total number of sections in which that nucleus appeared. The volume of the nucleus in each section was determined by multiplying the actual area of the nucleus by the thickness of the section (5u).

(2) Number of Neurons in Each Brain Nucleus: Niss1 and/or Masson Trichrome stained sections were used and the boundaries of the NOR, NPP and NLT were drawn on graph paper (details above) from every second or third section (10-15u intervals) at 950X magnification. Counts were made only for perikarya which contained a visible nucleus. Adjustments were made for double counting by applying the Abercrombie correction factor: P = A (M/L+M), where P = average number of nuclei per section, A = number of cell nuclei actually counted, M = thickness of section (5um), and L = average length of the cell nucleus (in um) (Abercrombie, 1946). To arrive at the total number of neurons in each brain nucleus, P was multiplied by the total number of sections in which that brain nucleus appeared.

(3) Number of Gonadotrops in Pituitary: Pituitaries prepared for immunocytochemistry were used for counting the number of gonadotrops according to the following procedures:

(a) Lateral Caudal Pars Distalis (lCPD): Nuclei of these gonadtrops were counted with an ocular reticule (900um^2) to provide the number of gonadtrops per unit area at a magnification of 950X. One section from each side of the lateral CPD, equidistant between the mid-sagittal and the extreme lateral plane, was chosen for each pituitary. Each section was carefully positioned under the net reticules so that the same area of the lCPD was evaluated in all pituitaries. Counts were made for only those ir-gonadotrops where the nucleus was visible.

(b) Ventral Caudal Pars Distalis (vCPD): A mid-sagittal section

was chosen and the total number of vCPD ir-gonadotrops in that section were counted.

(c) Pars Intermedia (PI): A mid-sagittal section was chosen and the total number of ir-cells were counted.

(4) Area of Neuronal Nuclei: Five to 10 neurons were randomly selected for calculating nuclear area using alternate sections from both hemispheres which contained the NOR, NPP or NLT. A total of 30-85 neurons of each region were chosen for measurement in each brain. The formula for the area of an ellipse was used, according to Morishita et. al., (1974), since each nucleus was essentially oval; area = $(\frac{\pi}{4})$ (ab) where "a" represents the greatest nuclear diameter and "b" the greatest diameter that is perpendicular to "a".

(5) Cellular and Nuclear Indices (C.I. and N.I.) of Neurons:

(a) Cellular Index: according to the method of Leatherland (1970). 5 - 10 perikarya were measured on alternate sections of each NOR, NPP and NLT, with an ocular micrometer at a magnification of 950X. A total of 30 to 85 of each type of neuron was measured and their dimensions expressed according to the following formula:

$$\text{C.I.} = \frac{\text{maximum cell length} + \text{maximum cell width}}{2}$$

(b) Nuclear Index: The procedure used in 5(a) discussed above was also used to calculate Nuclear Index (N.I.):

$$\text{N.I.} = \frac{\text{maximum nuclear length} + \text{maximum nuclear width}}{2}$$

(6) Cellular and Nuclear Indices of Gonadotrops and Pars Intermedia Cells:

(a) Cellular Index: The method of Leatherland (1970) used for calculating the cellular and nuclear indices of perikarya described above,

was similarly applied to ir-GTH containing cells. One mid-sagittal section and two lateral sections, each equidistant from the mid-sagittal section, were chosen to determine the dimensions of cellular and nuclear indices. Five - 10 gonadotropic cells and 5-10 PI cells were measured with an ocular micrometer at a magnification of 950X by placing the micrometer along the longest axis of the area containing the cells of interest. A total of 35-65 gonadotropic and/or PI cells were measured per pituitary gland.

Statistical Analysis:

Means and standard errors were calculated for each group of variables. One way analysis of variance (ANOVA) was used to test differences among more than two groups from which data had been obtained. If there were significant differences among the group means as indicated by the F test, a Newman-Keuls test (Zivin and Bartko, 1976) was performed to find out which pairs of means were significantly different. In addition, the Student's t test was applied. In all cases, significant difference between the groups was selected at less than the 5% level ($P < 0.05$). These methods were used in lieu of Duncan's multiple range analysis which had yielded similar results on evaluation of selected data.

Results

I. Ontogeny and Cytometry of LHRH Containing Regions in the Brain and Pituitary

Since no sexual dimorphism was noted in these studies, the description which follows applies to both male and female fish of either early ($\underline{P}^1\underline{P}^2$) or late ($\underline{P}^2\underline{P}^5$) maturing genotypes at all stages or ages of development indicated. The anatomical terms used are according to Peter et al., (1975), Kim et al. (1979) Nieuwenhuys (1982) and Münz et al. (1981). Figure 1 is a diagrammatic representation of a platyfish brain and Figure 2 depicts where immunoreactive (ir-) LHRH is found in the adult platyfish brain and pituitary gland. The specific staining to be described for anti-LHRH or anti-GTH was absent in all sections in which antiserum had been preabsorbed with corresponding antigens.

(A) Adult (Stage 6)

(1) Nucleus Olfactoretinalis (NOR)

The nucleus olfactoretinalis (NOR) comprises the most anterior group of LHRH containing neurons. It is located in each hemisphere of the brain in the rostral border of the area ventralis pars telencephali, close to the olfactory lobe and adjacent to the fissura circularis. The neurons of the NOR are fusiform in shape, giving them a bipolar appearance; they have limited basophilia, large, oval pale-staining nuclei and prominent nucleoli (fig. 3).

In early maturers ($\underline{P}^1\underline{P}^2$), the relative volume and indices of cellular and nuclear size of the NOR, reach maximum values at 20 weeks (stage 6) and remain unchanged throughout the course of this study (34 weeks for $\underline{P}^1\underline{P}^2$ fish) (see Plates 3-6 and tables 5 and 6).

In late maturers ($\underline{P}^2\underline{P}^5$), maximum values are attained when stage 6 is reached at 59 weeks of age (Plate 3, 7-9 and tables 5 and 6), and

these do not change with time (compare 59 and 73 week old $\underline{P}^2\underline{P}^5$ fish in tables 5 and 6), (see Plates 7-9).

ICC: In both $\underline{P}^1\underline{P}^2$ and $\underline{P}^2\underline{P}^5$ adults, perikarya contain less intense LHRH immunoreactivity than is present in earlier stages of development (see below). In addition, ir-LHRH containing processes which originate in the NOR, project in 3 directions; rostrally, along the ventral surface of the olfactory lobe (fig. 4), dorsocaudally toward the anterior commissure and pineal complex (fig. 4), and ventrocaudally into the optic tract and anteroventral portion of the nucleus preopticus periventricularis (NPP). Still other fibers continue from the NOR in association with projections from perikarya of the NPP toward the nucleus lateralis tuberis (NLT) and to the neurohypophysis of the pituitary, and to the mesencephalon.

(2) Anteroventral Nucleus Preopticus Periventricularis (NPP).

A second population of LHRH containing neurons, localized in the anteroventral portion of the nucleus preopticus periventricularis (NPP) (according to Peter et al., 1975, also called the nucleus preopticus basalis lateralis by Munz et al., 1981) form a border along the ventral surface of the rostral diencephalon, lateral and ventral to the nucleus preopticus (NPO). In sexually mature $\underline{P}^1\underline{P}^2$ fish these perikarya are quite basophilic, and contain an oval, pale staining nuclei and prominent nucleoli. At 20 weeks of age as $\underline{P}^1\underline{P}^2$ fish enter stage 6 volume and cellular and nuclear indices are significantly greater than those for $\underline{P}^2\underline{P}^5$ sibs at the same age but who are still in stage 1 (N.A., 23.0um^2, $\underline{P}^1\underline{P}^2$; 9.2um^2, $\underline{P}^2\underline{P}^5$ ($P < .02$); C.I., 5.5um, $\underline{P}^1\underline{P}^2$; 4.3um, $\underline{P}^2\underline{P}^5$ ($P < .03$); N.I., 5.3um, $\underline{P}^1\underline{P}^2$; 3.5um, $\underline{P}^2\underline{P}^5$ ($P < .04$); see Plates 3-9 and tables 5 and 7). When $\underline{P}^2\underline{P}^5$ fish reach stage 6, at 59 weeks of age, the morphometric values for the N.A., C.I. and N.I. of the NPP are then similar ($P < .05$)

to those of early-maturers at that stage and age.

ICC: Perikarya display an intense immunoreactive response with anti-LHRH (fig. 5) and ir-processes emanate in distinct fasicles that travel ventrocaudally just dorsal to the optic chiasm, and at the level of the horizontal commissure (HC) course ventrally towards the lateral and medial portion of the hypothalamus and the NLT. Some fibers enter the hypophysial stalk (fig. 6) and end near gonadotropic cells, while others continue along with fibers of the NOR toward the ventral mesencephalon where they become difficult to trace.

(3) Nucleus Lateralis Tuberis Pars Posterioris (NLT)

The third group of LHRH containing neurons are in the nucleus lateralis tuberis (NLT) which is located in the lateral and lateroventral walls of the hypothalamus. The perikarya of $\underline{P}^1\underline{P}^2$ and $\underline{P}^2\underline{P}^5$ fish are unipolar and contain basophilic cytoplasm that surrounds euchromatic nuclei with prominent nucleoli (fig.). In $\underline{P}^1\underline{P}^2$ animals the volume of the NLT is similar ($P < .05$) but its cellular and nuclear indices are significantly greater ($P < .04$) than $\underline{P}^2\underline{P}^5$ animals at the same stage (see Plates 3-9 and table 5 and 8).

ICC: Ir-LHRH perikarya in the NLT of $\underline{P}^1\underline{P}^2$ fish (fig. 7) appear as a cluster slightly dorsal and caudal to the posterior pituitary and show paler immunoreactivity than perikarya of the NOR and NPP at the same antiserum concentration. In adult $\underline{P}^1\underline{P}^2$ fish the number of ir-perikarya has decreased, when compared to stage 2 (to be discussed; see table 9). Processes form a "netted" pattern around these perikarya, with no apparent direction. No ir-LHRH perikarya are noted in $\underline{P}^2\underline{P}^5$ fish at stage 6, although ir-fibers are visible.

(4) Pituitary:

(a) Gonadotrop (GTH) Zone of the Caudal Pars Distalis (CPD):

The adult pituitary has been previously described by Schreibman (1964). In all sexually mature platyfish, the gonadotrops which form the periphery of the CPD, are irregular in shape, stain light blue to purple, and contain pale staining irregular shaped nuclei and a single eccentric, dark staining nucleolus (fig. 8).

In $\underline{P}^1\underline{P}^2$ fish, the cell and nuclear indices are similar (P=.05) and the total number of ir-gonadotrops in the vCPD are significantly greater ($P < .03$) than those of late-maturing sibs (Plates 10-12 and tables 9,10).

ICC: Both ir-LHRH (fig. 8) and GTH can be seen within all CPD gonadotrops but at higher magnifications, only ir-LHRH, and not GTH, is visible between gonadotrops. In both early and late maturers, GTH always stains more intensely than LHRH in the pituitary.

(b) Pars Intermedia (PI):

In the PI, cells that contain ir-LHRH and ir-GTH are round to oval and their basophilic cytoplasm surrounds a round to oval-shaped pale staining nucleus and a centrally placed dark staining nucleolus. These cells are also periodic acid Schiff positive (PAS+) (Schreibman and Margolis-Kazan, 1979). No significant differences ($P < .05$) are noted in the cellular and nuclear indices of these cells in $\underline{P}^1\underline{P}^2$ and $\underline{P}^2\underline{P}^5$ fish at stage 6 (Plate 13 and table 11).

ICC: The total number of cells containing ir-LHRH and ir-GTH in the PI are similar ($P = .05$) in $\underline{P}^1\underline{P}^2$ and $\underline{P}^2\underline{P}^5$ fish (Plate 10 and table 9).

(B) One Week Old Fish:

(1) NOR:

Perikarya of this region are bipolar in appearance and have a

thin rim of basophilic, an oval, euchromatic nucleus, and a central prominent nucleolus (fig. 9). Several processes emanate in all directions from the cell bodies. No significant differences ($P > .05$) were observed between early and late maturing genotypes for any of the morphometric parameters examined (Plates 3-9 and tables 5, 6).

ICC: Ir-LHRH was not observed in perikarya or axons in either genotype at 1 week of age.

(2) NPP:

The NPP consists of perikarya separated by a small amount of neuropil. These neurons are amorphic to ovoid in shape and have a thin rim of fuchsinophilic cytoplasm that surrounds a densely stained nucleus. A single nucleolus can be seen in only some cells. The processes from these perikarya could not be traced. No significant differences ($P > .05$) between early and later maturers were observed for any of the cytometric parameters in the NPP at this age (see Plates 3-9 tables 5, 7).

ICC: No ir-material is seen in perikarya or axons of the NPP at one week of age.

(3) NLTp:

Neurons of the NLT appear irregular in shape in week old fish. With Masson's stain the perikarya contain a thin rim of red to pink cytoplasm which encloses a densely stained nucleus and no nucleoli can be distinguished. A small amount of neuropil separates these neurons from the rest of the NLT and axonal projections are not seen at this stage. Morphometric values are comparable for C.I. and N.I. in $\underline{P}^1\underline{P}^2$ and $\underline{P}^2\underline{P}^5$ sibs ($P > .05$) (see Plates 3-9 and tables 5,8).

ICC: No ir-material is observed in perikarya or axons in either genotype at one week of age.

(4) Pituitary Gland:

a) CPD: The pituitary of one week old fish, regardless of genotype, is a flattened ellipsoid-shaped structure closely attached to the floor of the diencephalon by a thin-walled stalk. A mid-sagittal section shows the hypophysis to be composed of equal areas of adeno- and neurohypophysial tissue (see Schreibman, 1964).

The vCPD destined to become the GTH zone, consists of a few chromophobic cells with amorphic-shaped, densely stained nuclei. Nucleoli are not evident. Cellular and nuclear indices are comparable ($P > .05$) in $\underline{P}^1\underline{P}^2$ and $\underline{P}^2\underline{P}^5$ fish (Plate 12 and table 10).

ICC of the vCPD: Ir-LHRH and ir-GTH are present in limited amounts in the cytoplasm of the few scattered cells of the vCPD (fig. 10).

Sections through the lateral CPD (lCPD) of the GTH zone show a different cytological profile than those through the vCPD. Anilin blue cells form clusters on each side of the CPD. Their number, cellular and nuclear indices do not vary significantly ($P \geq .05$) between genotypes (Plate 11 and table 10).

ICC of the lCPD: Both ir-LHRH and ir-GTH (fig. 10, 11) are present in these cells.

b) PI: The ir-GTH positive cells in the PI are arranged in clusters and contain round to oval pale staining nuclei and single nucleoli.

ICC: These cells show more intense immunoreactivity with both anti-cGTH (fig.11) and anti-LHRH.

(C) Stage 1 to Stage 2:

(1) NOR:

a) Five weeks:

At 5 weeks of age (stage 1) the perikarya of $\underline{P}^1\underline{P}^2$ fish are bipolar in shape and contain pale staining nuclei and single nucleoli and basophilia that is more pronounced than is seen at one week (fig. 12). Perikarya of $\underline{P}^2\underline{P}^5$ fish are fusiform in shape, lightly basophilic, and have a euchromatic nucleus and a centrally placed nucleolus. All morphometric values are signficantly greater ($P < .05$) in $\underline{P}^1\underline{P}^2$ fish than in $\underline{P}^2\underline{P}^5$ (see tables 5,6; N.A.; $\underline{P}^1\underline{P}^2$, 14.1um^2; $\underline{P}^2\underline{P}^5$, 11.1um^2 ($P < .03$); C.I.; $\underline{P}^1\underline{P}^2$, 5.3um; $\underline{P}^2\underline{P}^5$, 4.5um ($P < .04$); N.I.; $\underline{P}^1\underline{P}^2$, 4.5um; $\underline{P}^2\underline{P}^5$, 4.0um ($P < .05$).

ICC: a) 5 weeks ir-LHRH is present in all perikarya of the NOR of early maturers (fig. 12) as well as in their processes which project caudally towards the ventral diencephalon. The NOR of late maturers does not contain ir-LHRH in perikarya or fibers at this age.

b) 11 - 25 weeks: At 11 weeks, there are larger increases in nuclear area and cellular and nuclear indices, in the NOR of both $\underline{P}^1\underline{P}^2$ animals (entering puberty) and their $\underline{P}^2\underline{P}^5$ sibs (still in stage 1) (Table 6). From 11 to 25 weeks the perikarya in the NOR of $\underline{P}^2\underline{P}^5$ animals show an increase in basophilia, and contain larger, pale nuclei and single nucleoli. In addition, all cytometric values have increased (Plates 3, 7-9 and tables 5,6).

ICC: At 11 weeks in $\underline{P}^2\underline{P}^5$ fish, ir-material is present in NOR. perikarya and pathways of axons are easily traced because of their more pronounced ir-response. At 18 weeks, however, there is a marked increase in the amount of ir-material which is greatest at 25 weeks.

(2) MPP:

$\underline{P}^1\underline{P}^2$ fish contain NPP perikarya that are ovoid, have lightly basophilic, and euchromatic nuclei with centrally placed nucleoli. $\underline{P}^2\underline{P}^5$ sibs have perikarya with less basophilic cytoplasm, more densely stained nuclei and no prominent nucleoli. At five weeks of age, cytometric measurements for $\underline{P}^1\underline{P}^2$ (stage 1) fish have increased significantly compared to their stage 1 (also 5 week old) $\underline{P}^2\underline{P}^5$ sibs (N.A., 8.8um^2, $\underline{P}^1\underline{P}^2$; 7.2um^2, $\underline{P}^2\underline{P}^5$ ($P < .04$); C.I. 4.1um, $\underline{P}^1\underline{P}^2$; 3.6um, $\underline{P}^2\underline{P}^5$ ($P < .05$); N.I., 3.6um, $\underline{P}^1\underline{P}^2$; 3.2um, $\underline{P}^2\underline{P}^5$ ($P < .05$); see Plates 3-9 and tables 5,7).

In $\underline{P}^1\underline{P}^2$ fish, there is a consistent, marked increase in cytometric values from birth up to 11 weeks of age (stage 2). In $\underline{P}^2\underline{P}^5$ sibs there is a more gradual increase from birth up to 26 weeks of age which coincides with their reaching stage 2 (see Plates 3-9 and tables 5,7).

ICC: No ir-perikarya are present in either genotype in stage 1. Ir-fibers are present in the NPP of $\underline{P}^1\underline{P}^2$ fish at 5 weeks, but they do not apppear in $\underline{P}^2\underline{P}^5$ animals until 11 weeks of age.

(3) NLT

At 5 weeks of age, NLT perikarya of Masson stained $\underline{P}^1\underline{P}^2$ fish contain a thin rim of pink cytoplasm, nuclei that appear less dense, and visible nucleoli. $\underline{P}^2\underline{P}^5$ fish contain NLT cell bodies which differ from $\underline{P}_1\underline{P}^2$ sibs in that the cytoplasm is more fuchsinophilic, the nuclei are more dense and nucleoli are not visible. All cytometric parameters in $\underline{P}^1\underline{P}^2$ and $\underline{P}^2\underline{P}^5$ sibs increase between 1 and 5 weeks of age. However, at stage 1, cytometric values for early maturers are significantly larger than those for late maturing sibs (N.A., 6.3um^2, $\underline{P}^1\underline{P}^2$; 5.2um^2, $\underline{P}^2\underline{P}^5$ ($P < .04$); C.I., 3.4um, $\underline{P}^1\underline{P}^2$; 2.9um, $\underline{P}^2\underline{P}^5$ ($P < .05$); N.I., 3.0um, $\underline{P}^1\underline{P}^2$; 2.2um, $\underline{P}^2\underline{P}^5$ ($P < .05$); see Plates 3-9 and tables 5,8).

ICC: No ir-perikarya are present in either genotype. However,

ir-material is seen in a limited number of fibers in the NLT of only $\underline{P}^1\underline{P}^2$ fish, but not $\underline{P}^2\underline{P}^5$ fish.

(4) Pituitary: CPD and PI

a) 5 weeks:

The developmental picture of the hypophysis of $\underline{P}^1\underline{P}^2$ and $\underline{P}^2\underline{P}^5$ fish in stage 1 is similar to that seen at one week of age except that the pituitary gland is larger, and the neurohypophysial tissue has begun to extend into the adenohypophysis.

When viewed in cross section, the pituitary gland of both $\underline{P}^1\underline{P}^2$ and $\underline{P}^2\underline{P}^5$ fish, contains a cluster of anilin blue positive cells in each of the two extreme lateral regions of the gland. Along the ventral border, a single layer of chromophobic cells extends between these clusters. In $\underline{P}^1\underline{P}^2$ fish, the cellular and nuclear indices of the lCPD and vCPD are all significantly greater ($P < .05$) than those of their $\underline{P}^2\underline{P}^5$ sibs (see Plates 11,12 and table 10).

At stage 1, the PAS+ cells of the PI appear similar. The C.I., N.I. and number of ir-GTH and ir-LHRH containing cells have increased similarly ($P = .05$) in both early and late maturers (Plate 13 and table 11).

ICC of the CPD: In $\underline{P}^1\underline{P}^2$ fish, the number of ir-cells in both the lCPD and vCPD are greater than in $\underline{P}^2\underline{P}^5$ sibs (Table 9). In addition, in both genotypes the cells of the lCPD are more intensely immunoreactive than those in the vCPD.

ICC of the PI: The number of ir-GTH and LHRH containing cells have increased similarly in both genotypes (see Plate 10).

b) 11-25 weeks

In $\underline{P}^1\underline{P}^2$ fish, the hypophysis has undergone dramatic

developmental changes between the time of stage 1 (5 weeks of age), and the onset of stage 2 (10-11 weeks of age). The lCPD and vCPD now consist of a thin layer of basophils which have greatly increased in number (5-6 fold) (Plate 10 and table 9), contain oval to irregularly shaped nuclei and single nucleoli. There is an increase in the cellular index of the lCPD and vCPD. The nuclear index of the lCPD gonadotrops are similar at 5, 10 and 11 weeks, but the vCPD nuclear index has increased significantly ($P < .05$) (see Plates 11, 12 and table 10).

In $\underline{P}^2\underline{P}^5$ fish from stage 1 (5 weeks of age) to the onset of stage 2 (25-26 weeks), the hypophysis contains significantly ($P < .02$) fewer cells in the lCPD and vCPD when compared to P^1P^2. Some cells in the lCPD are basophilic and those in the vCPD are chromophobic. The nuclei of the cells in both regions appear less dense and some nucleoli can be discerned. There are increases in the cellular and nuclear indices of the lCPD and vCPD of $\underline{P}^2\underline{P}^5$ fish from 5 to 26 weeks (Plates 11, 12 and table 10).

The number of cells in the PI and their cellular and nuclear indices are similar in both genotypes (Plate 13 and table 11).

ICC: In $\underline{P}^1\underline{P}^2$ fish, an increase ($P < .03$) in the number ir-GTH containing cells in the lCPD and vCPD is noted between 5 and 11 weeks (table 9) and ir-GTH staining is significantly more intense than that of ir-LHRH.

In $\underline{P}^2\underline{P}^5$ fish, as in early maturers, ir-GTH stains more intensely than ir-LHRH in the lCPD and vCPD cells.

The ir-cells of the PI of both $\underline{P}^1\underline{P}^2$ and $\underline{P}^2\underline{P}^5$ fish have increased similarly in number from stage 1 to stage 2 (Plate 10 and table 9).

(D) Stage 2 to Stage 6:

(1) NOR:

At stage 2, perikarya of $\underline{P}^1\underline{P}^2$ fish are basophilic, contain pale nuclei and a more prominent nucleoli. Nuclear area and cellular and nuclear indices in stage 2 early maturers (N.A., 17.8um^2; C.I., 6.3um; N.I., 5.2um) have increased since stage 1. The NOR volume is also significantly greater ($P < .05$) than in stage 1 (see figs. 3-9 and tables 5, 6).

The cytometric dimensions for the NOR of stage 2 $\underline{P}^2\underline{P}^5$ fish (N.A., 22.1um^2; C.I., 6.8um; N.I., 5.5um) are significantly greater ($P < .04$) than those of $\underline{P}^1\underline{P}^2$ sibs at the same stage. The volume is also significantly greater (see figs. 3-9 and tables 5, 6).

ICC: At stage the amount of ir-LHRH contained in perikarya (fig. 13) and fibers has increased in both genotypes and pathways of processes from the NOR can now be easily followed.

(2) NPP

In $\underline{P}^1\underline{P}^2$ fish, the NPP now contains bipolar, more basophilic perikarya with pale staining nuclei and centrally placed. All measurements have increased ($P < .05$) above those observed at stage 1 (figs. 3-6 and table 5, 7). Significant differences ($P < .05$) in all cytometric parameters are found between $\underline{P}^1\underline{P}^2$ and $\underline{P}^2\underline{P}^5$ fish in stage 2 (figs. 3-9 and tables 5, 7).

ICC: Both genotypes contain ir-LHRH positive perikarya, whose processes begin to form a fasicle (fig. 14) that extend to more caudal regions of the diencephalon. A greater number ($P < .03$) of NPP ir-perikarya and fibers are noted in $\underline{P}^1\underline{P}^2$ fish as compared to $\underline{P}^2\underline{P}^5$ animals from stage 2 to 6 (Plate 10 and table 9).

(3) NLT

$\underline{P}^1\underline{P}^2$ fish contain NLT perikarya with euchromatic nuclei, single nuclei and lightly stained basophilia situated mainly towards one pole. The NLT of $\underline{P}^2\underline{P}^5$ fish contains perikarya with lightly basophilic cytoplasm which surrounds a denser nucleus with a pale nucleolus. Signficant differences ($P<.04$) are seen in all cytometric values when $\underline{P}^1\underline{P}^2$ and $\underline{P}^2\underline{P}^5$ fish of the same stage are compared (see figs. 3-9 and table 5, 8).

ICC: Perikarya and processes in the NLT of $\underline{P}^1\underline{P}^2$ fish contain ir-material (fig. 15) and fibers form a network in proximity to the cell bodies. In $\underline{P}^2\underline{P}^5$ fish ir-LHRH accumulates only in fibers but not perikarya of the NLT.

(4) Pituitary

a) CPD

In stage 2, $\underline{P}^1\underline{P}^2$ fish, the gonadotropic zone consists of 2-3 lays of granular cells, with pale nuclei, single prominent red nucleoli and cytoplasm which is intensely stained with anilin blue (Massen's trichrome). The gonadotrops of stage 2, $\underline{P}^2\underline{P}^5$ fish contain a narrow rim of cytoplasm which stains pale with anilin blue, a densely stained nucleus and a pale nucleolus. Results for all cytometric parameters measured in $\underline{P}^2\underline{P}^5$ fish are significantly ($P<.05$) lower than those observed in early maturers (Plates 11, 12 and table 10).

ICC: The number of ir-LHRH and ir-GTH gonadotrops in $\underline{P}^1\underline{P}^2$ fish have already increased significantly ($P<.02$) at the onset of stage 2 (see Plate 10 and table 9). This number of ir-cells is significantly greater ($P<.02$) than is seen in $\underline{P}^2\underline{P}^5$ animals at a comparable stage. This significant difference between early and late maturers continues into adulthood (see Table 9).

b) PI

The PI cells of both genotypes at stage 2 are spindle-shaped,

contain basophilic cytoplasm, pale blue nuclei and prominent red nucleoli. There are no apparent differences ($P > .05$) between $\underline{P}^1\underline{P}^2$ and $\underline{P}^2\underline{P}^5$ fish in all cytometric values, from stage 2 to stage 6 (adulthood) (Plate 13 and table 11).

ICC: There is little difference ($P = .05$) between genotypes in the number of ir-GTH and ir-LHRH PI cells (Table 9).

II. The Effect of Hypophysectomy (H) on the Brain of Sexually Mature Platyfish

A. H for one week:

(1) NOR

In the NOR of fish hypophysectomized for one week, Nissl stained sections depict perikarya which contain light basophilia surrounding euchromatic nuclei and prominent nucleoli. Values for all morphometric parameters measured in the NOR of H and sham-operated animals are similar ($P > .05$; Plate 14 and table 12).

ICC: There is little change in content perikarya and processes in fish H for one week.

(2) NPP

Perikarya in the NPP contain a deeper basophilia that surrounds a pale nucleus and a single nucleolus. Cellular indices are significantly decreased ($P < .05$) H fish as compared to sham-operated ones (see Plate 14 and table 12).

A total depletion of ir-LHRH is seen in perikarya but not in processes of this brain region when fish are hypophysectomized for 1 week.

(3) NLT

Neurons appear hypertrophied and contain perikarya with deep

blue basophilia, very pale nuclei and more prominent centrally placed nucleoli. The cellular and nuclear indices of H fish are significantly greater ($P < .05$) than observed for sham-operated fish (see Plate 14 and table 12).

ICC: Ir-material has disappeared in perikarya of the NLT and only a few ir-fibers are now seen.

B. H for three weeks:

(1) NOR

Fusiform-shaped intensely basophilic perikarya surround euchromatic nuclei and prominent nucleoli. Significant increases ($P < .05$) in cellular measurements are seen when 3 week H fish are compared to 1 week H and sham-operated groups (see Plate 14 and table 12).

ICC: The amount of ir-LHRH has slightly increased in perikarya and fibers of the NOR.

(2) NPP

Cells are oval with blue to deep purple perikarya which surround euchromatic nuclei and prominent nucleoli. The cellular and nuclear indices for the NPP are significantly increased ($P < .05$) when 3 week H fish are compared to fish H for 1 week (Plate 14 and table 12).

ICC: Although ir-material is visible in a few processes of 3 week H fish, no ir-perikarya are seen in the NPP.

(3) NLT

Irregular shaped perikarya contain deep blue basophilia that surrounds a very pale nucleus with a nucleolus which appears enlarged. The cellular and nuclear indices have increased ($P < .05$) compared to fish H for 1 week (Plate 14 and table 12).

ICC: Immunoreactive material is now absent from processes, as well as perikarya.

C. H for five weeks

(1) NOR

Fusiform-shaped perikarya are larger and more basophilic, pale staining nuclei are enlarged, and nucleoli are more intensely stained when compared to the other experimental groups (fig .16 and table 12).

ICC: Between 3 and 5 weeks there is a dramatic increase in the intensity of ir-LHRH that accumulates in perikarya and processes of the NOR.

(2) NPP

Oval perikarya are basophilic, nuclei are pale and nucleoli appear enlarged. No significant differences ($P > .05$) are evident in the cellular and nuclear indices when animals H for 5 weeks are compared to animals H for 3 weeks (Plate 14 and table 12).

ICC: Although a few fibers contain ir-material, no ir-LHRH is localized in perikarya of the NPP.

(3) NLT

Irregular shaped perikarya contain deep basophilia, prominent nuclei and nucleoli. No significant differences ($P > .05$) in the cytometric parameters are noted when animals H for 5 weeks are compared to animals H for 3 weeks (Plate 14 and table 12).

ICC: As in fish H for 3 weeks, no ir-material is evident in perikarya or fibers of the NLT.

III. Effect of and gonadotropin administration on the distribution and quantity of LHRH in the brains of sexually mature platyfish H for 5 weeks.

(1) Group 1: Sham-operated-saline injected:

The neurons of the NOR, NPP and NLT contain lightly basophilic

rings of cytoplasm that surround euchromatic nuclei and single centrally placed nucleoli. There are no significant differences ($P > .05$) between sham-operated animals at 1, 3 and 5 weeks and sham-operated animals that received saline when all cytometric measurements are compared (Plate 14 and table 12).

ICC: Ir-LHRH is localized in perikarya and processes of the NOR (fig. 16), NPP and NLT. As in intact animals, reaction intensity is greatest in the perikarya of the NPP, less in the NOR and least in the NLT. Within the hypophysis ir-LHRH is localized within GTH cells of the CPD, and within processes between these cells, and in the PAS+ cells of the PI

RIA: An average value of 649 $\pm$ 28 picograms of LHRH per brain was determined for the eight brains assayed. A total value of 507.5 picograms of LHRH was assayed in the eight pooled whole pituitary glands (Table 12).

(2) Group II: Hypophysectomized-saline-injected:

Perikarya in the NOR, NPP and NLTp are more intensely stained with anilin and methyline blue, nuclei appear paler and nucleoli are more prominent compared to sham-operated fish. All cytometric parameters are significantly greater ($P < .05$) when compared to control group values (see Plate 14 and table 12).

ICC: Brains of animals hypophysectomized for five weeks contain a marked reduction of ir-LHRH content in the NPP and NLTp. There is a total depletion of ir-material in the perikarya of these regions, however, a few fibers emanating from the NPP and NLTp do show immunoreactivity. In the NOR, there is a marked increase in immunoreactive staining in both perikarya and fibers (fig. 17).

RIA: An average value of 446 $\pm$ 31 picograms of LHRH per brain

was calculated for the twelve brains assayed (Plate 15 and table 12). This is significantly less ($P<.03$) than in sham-operated fish (649 ± 28).

(3) Group III: Hypophysectomized GTH-injected:

Animals treated with salmon GTH (SG-G100) contain perikarya in the NOR that are less basophilic than are noted in the NOR of H-saline-injected animals. Nuclei have decreased in size and nucleoli resemble those seen in sham-operated controls (Plate 14 and table 12). In the NPP, neurons contain lighter basophilia than seen in H-saline injected fish and oval pale nuclei and prominent nucleoli. In the NLT, perikarya still contain a deep blue basophilic ring of cytoplasm, pale nuclei, and prominent nucleoli. A decrease in all morphometric parameters ($P < .05$) is noted when compared to values depicted in H-saline-injected.

ICC: A slight increase in the number of ir-LHRH containing neuronal fibers is noted in the NPP and NLTp of fish receiving salmon GTH. In the NOR, however, although there is a marked decrease of ir-material in perikarya and fibers, compared to H-saline-injected fish, there is still considerably more than is noted in sham-operated groups (compare figs. 17 and 18).

RIA: An average of 362 ± 36 picograms of LHRH per brain was found in the eleven brains assayed. This value is significantly different from animals in Group I ($p < 0.05$) and Group II ($p < 0.05$) (Plate 15 and table 12).

GONADS:

(1) Sham-Operated Fish:

All ovaries of sham-operated fish contained many large yolky oocytes. Few oogonia or primary oocytes were present.

(2) Hypophysectomized - Saline Injected Fish:

Ovaries in 75% of the fish hypophysectomized for 1,3, or 5 weeks showed a small degree of oocyte atresia in addition to a ponderence of large yolky oocytes.

(3) Hyopphysectomized - SG-G100 Injected Fish:

Approximately 75% of the GTH-injected animals lacked yolky oocytes. Few oogonia and many primary oocytes in the oil droplet stage were evident.

Discussion -

I. Methods:

Bouin's fluid containing acetic acid was chosen for all histological and immunocytochemical studies since it has been clearly established to provide good preservation of certain antigens with immunocytochemical methods (Sternberger, 1979). As with all chemical fixatives, this solution causes some shrinkage in tissues. However, since Bouin's was the only fixative used throughout this study, the shrinkage error that might have been introduced would have remained uniform throughout the various experiments and, therefore, would not be expected to affect the relative results (Bereiter and Jeanrenaud, 1974).

The ability to assess the functional state of secretory cells at the light microscope level has been discussed in detail by Schreibman and Holtzman (1975). Changes in the distribution, number, size and extent of development of the organelles reflect the activity of a cell. At the light microscope level, a decrease in the secretory product can be seen by a reduction in the intensity of stain in the cell and indicates that secretion is taking place. An increase in the cell size, nuclear volume and in the prominence and staining affinity of the nucleolus reflects transcriptional activity. An increase in the basophilia of the cell, which is easily evaluated by Nissl stain, indicates an increase in ribosomal material. If, however, there is a diminution in the demand for protein product, then there is a concomitant decrease in the structural components involved in the transcription, translation, and transport phases that are part of the formation of the secretory substances and thus a reduction in Nissl staining. Obviously, one cannot rely solely on these morphological and tinctorial parameters for a total evaluation of a cell's performance in response to changes in its internal and external

environment. As with all techniques, there are shortcomings and histological methods are no exception. To augment the histological and cytological approach, therefore, this study has utilized immunocytochemical methods to evaluate the presence of specific protein products (LHRH and GTH) in fish in various physiological states of their reproductive systems.

To determine the volume of each LHRH-containing region of the brain, the number of sections drawn with the camera lucida was increased over that recommended by Schwanzel-Fukuda et al. (1981), in order to reduce sampling error (Zilles et. al., 1982).

The use of nuclear area, nuclear and cellular indices and number are well accepted procedures for analyzing changes in cellular activity (Leatherland and Ensor, 1973; Morshita et al., 1974; Holtzman, 1975; Fahraeus-van Ree et al., 1983). Random samples of the cellular population were chosen in order to avoid subjectivity of choice and adjacent sections were carefully analyzed in order to preclude duplicate measurements or counts of the same cells. For cellular density measurements the mid-sagittal sections and sections equidistant from the lateral boundary and the mid-sagittal plane of the pituitary, were carefully determined to avoid bias in selection.

The LHRH antibodies utilized for ICC and RIA analyses in this study were prepared against the C-terminus of synthetic LHRH (Goos et al., 1976; Barry, 1979). It is interesting to note that antisera to purified mammalian LHRH, also prepared against the C-terminus (Arimura-743), crossreacts in the same platyfish brain and pituitary regions as the anti-synthetic LHRH (Goos, et al., 1976) used in the ICC portion of this study. Recently, Sherwood, et al. (1983) have shown that salmon and mammalian LHRH differ at the seven and eight positions. Therefore, when

using heterologous antisera it is important to correlate immunocytochemical results with physiological data, as was done in this study.

Several teleost gonadotropins and their alpha and beta subunits have been prepared in a highly purified state, thereby allowing the preparation of antibodies (Burzawa-Gerard et al., 1976). Carp gonadotropin (C-GTH) and salmon gonadotropin (SG-G100) are glycoprotein in nature and have similar molecular weights to the mammalian gonadotropins (Fontaine and Burzawa-Gerard, 1978; Idler and Ng, 1983). Although identical alpha subunits are present in TSH and the gonadotropins, it is the beta subunit that gives the glyprotein hormones their biological specificity and function. The antigens SG-G100, GTH and GTHB, as well as antibodies to them, are biologically active in teleosts (van Oordt and Peute, 1983; Idler and Ng, 1983), thus justifying the use of the GTH antiserum employed in this study.

The perikarya, fibers or pituitary cells which exhibited cross-reactivity with anti-LHRH or anti-GTHB were not "stained" when many of the "control" solutions were employed. These results demonstrate the specificity of both the method and the antibodies employed in this study. Method specificity deals with tissue components staining with reagents other than the antibodies. The omission and/or substitution of each reagent (discussed in Materials and Methods) and a series of dilutions of primary antiserum should determine the degree of method specificity (Childs, 1983; Petrusz, 1983; Pool et al., 1983). The determination of antibody specificity is based on the principal that antibodies can recognize short amino acid sequences on larger antigen molecules. Absorbing the antisera with their respective antigens tests the ability of the antibodies to recognize a specific amino acid sequence in the

antigen, and immunostaining will be prevented if this has occurred. However, the antibody may combine with impurities in the antigen so that the validity of the absorption test also depends upon the purity of the antigen (Pool et al., 1983). Only highly purified antigens (synthetic LHRH, Peninsula Laboratories, and cGTH and cGTHB, Burzawa-Gerard, 1982) were used for absorption in this study.

RIA determinations and ICC localizations, per se, have no bearing on the presence or absence of biologic activity associated with the antigen (Petrusz, 1983; Childs, 1983). All references to immunoreactive (ir-) LHRH and ir-GTH are really indicating "LHRH-like" and "GTH-like" material. It is, therefore, necessary to conduct physiological studies in addition to immunological methods to indeed show that the immunoreactive molecules being localized are biologically active (Hutson et al., 1979; Swaab, 1982; Petrusz, 1983), and this study, which examined animals in different physiological states (developmental stages and hypophysectomy), fulfills this condition.

II. Ontogeny and Functional Significance of Centers in the Brain and Pituitary that Contain Immunoreactive (ir)-LHRH

The ontogeny of LHRH containing centers in the brain was investigated in platyfish "genetically programmed" to reach puberty at two different ages (table 2). The results indicate that there is a sequential development of the three ir-LHRH containing areas in the brain that is directly related to stage of sexual development and not chronological age. The NOR is the first region to contain ir-LHRH which then appears in the NPP followed by the NLT. Anatomically, the sequence of this pattern of development proceeds from anterior to posterior and is essentially similar in both early and late maturing genotypes. Numerous

fibers emanate in all directions from the NOR and processes can be clearly delinated between this and the other nuclei. This phenomenon has been termed the "cascade effect" because of the apparent continuum in the pattern of development of the NOR, NPP, NLT and pituitary.

The NOR of early and late maturers increases in volume, nuclear area, and cellular and nuclear indices from one (neonatal) to five weeks (stage 1 gonopodial development). Ir-LHRH first appears in NOR perikarya at 5 weeks of age in early maturers, and 6 weeks later (11 weeks old and still in stage 1), in late maturers. Ir-cell bodies are at their maximum number when they first appear, and this number, which is similar in both genotypes remains constant into adulthood (see Plate 10). There are, however, significant increases in the dimensions of the NOR and of its individual perikarya in both genotypes from stage 1 to stage 2, a period that is characterized by the appearance of ir-fibers between the NOR and the NLT and NPP. These observations suggest increased cellular activity, a concept which is supported by cytological observations of increased basophilia and the appearance of pale nuclei and prominent nucleoli.

In the NPP, the overall volume and the dimensions of perikarya show substantial increases, in both early and late maturers, subsequent to those shown by the NOR. The NPP increases seen at stage 2, which occur in both genotypes, correspond with the initial appearance of ir-NPP perikarya. By stage 6 the NPP of both genotypes attains its maximum number of ir-perikarya, with early maturers containing approximately fifty percent more than late maturing sibs.

The dimensions of the NLT and its perikarya follow a similar pattern of change in both genotypes, and in this brain region measurements increase up to stage 2 and then decrease from stage 2 to 6. Ir-LHRH fibers first appear in stage 2 in both genotypes; however, although ir-

perkarya begin to appear shortly thereafter in early maturers, cell bodies with ir-LHRH are never seen in the NLT of late maturing sibs.

Other essential differences also exist between the genotypes studied. In the late maturers, specific steps of the "cascade effect" take place at similar developmental stages, but at different ages (older animals) and require more time to reach the full immunoreactive appearance characteristic of mature fish. This delay in time creates significant differences in the cytometric, cytologic, and immunologic characteristics of the three brain regions in fish of the same age but different genotype. For instance, at 5 weeks of age the perikarya in the NOR of early maturers contain ir-material and cytological analysis suggests that these cell bodies manifest secretory activity, i.e., pale nuclei, prominent nucleoli and cytoplasmic basophilia. Late maturers at the same chronological age lack ir-LHRH in perikarya and processes, their cytometric measurements are significantly smaller ($P < .05$), the perikarya are less basophilic, and nuclei appear dense. It is not until six weeks later (11 weeks, stage 1) that this genotype contains ir-LHRH perikarya and axons in the NOR; their cytometric measurements are now comparable to early maturers who, although they are the same chronological age, are now in stage 2. Also, the increase in size of the NOR from stage 1 to stage 2 is gradual in early maturers and "dramatic" in late maturers, so that at stage 2 all morphometric values for the NOR of late maturers are signficantly larger than those for early sibs.

The perikarya of the NPP and NLT appear to become more basophilic from stage 1 to stage 2, and show dramatic increases in all cytometric measurements. At stage 2, these characteristic increases are associated with the appearance of ir-perikarya in both the NPP and NLT of early maturers, but only in the NPP of late maturers. Also, a significantly

greater number of ir-LHRH perikarya are seen in the NPP of early maturers when compared to late maturers (Plate 10). In the NLT, early maturers also contain the maximum number of ir-perikarya and fibers at stage 2 (Plate 10), while late maturers contain ir-LHRH only in fibers but not in perikarya at this or any other stage. At stage 2 the NLT perikarya of $\underline{P}^2\underline{P}^5$ fish appear less basophilic and the nuclei are not as pale when compared to early $\underline{P}^1\underline{P}^2$ sibs.

The dimensions of the NPP in both genotypes increases dramatically from stage 2 to stage 6. The number of ir-LHRH NPP perikarya also increases by fifty percent in both genotypes but early maturers contain significantly more ($P < .03$) ir-cells than their late maturing sibs (Plate 10). By comparison the number of ir-LHRH perikarya in the NLT of early maturers has decreased by 50% from stage 2 to stage 6. However, although late maturers continue to lack NLT ir-perikarya, ir-fibers persist at stage 6.

The development of these three "LHRH-like" containing centers in the brain is directly related to events occurring in the pituitary gland and gonads. In all one week-old fish, regardless of genotype, ir-LHRH and ir-GTH are found in cells of the lCPD, scattered cells on the ventral border of the CPD and in PAS^+ cells of the pars intermedia. At this age ir-LHRH is not seen in the brain, however, this does not necessarily mean that the brain is not producing any. The presence of ir-LHRH in the pituitary of one week-old fish suggests that ir-LHRH production is occurring in the brain, however, the quantity may be insufficient to be detected by ICC methodology (Bigbee et al., 1977; Sternberger, 1979). One may also contemplate the possibility that the pituitary ir-LHRH observed is being produced by the ir-PI or -CPD cells, although, there is no evidence to support this hypothesis.

The possible role in reproductive function played by the immunoreactive cells of the pars intermedia in platyfish is suggested by their histochemical characteristics and by their life history. These cells, which are PAS^+, are also characterized by the presence of ir-GTH, -LHRH and their ability to bind sex steroids (Schreibman et al., 1982). In platyfish of both genotypes, ir-PI cells are present in neonates in a number that shows only a gradual increase into adulthood. Also, unlike the ir-CPD cells of early and late maturers, the total number of ir-PI cells is similar at each stage of development in both genotypes. These observations suggest that these cells play a role in the early maturation of the BPG axis, perhaps in collaboration with the cells of the lateral CPD, and that this role is probably similar in both genotypes. The role they play may be specifically related to the early development of the gonads, which, in neonatal teleosts, are characterized by the presence of enzymes necessary for synthesizing steroids and early stages of gametogenesis (Schreibman et al., 1982b; van den Hurk, 1974, 1983). These observations lend support to the concept that two types of gonadotrops are present in the platyfish pituitary gland (Schreibman and Margolis-Kazan, 1979), one type (PI) concerned with early and another (vCPD) with late maturational events.

The question of the number of gonadotrop types present in the teleost pituitary gland has been debated for many years. The idea that there are indeed two types of gonadotrops is gaining support due to recent observations in other teleosts (Ekengren et al., 1978; Olivereau and Olivereau, 1978; Borg, 1978; van Oordt and Peute, 1983; Idler and Ng, 1983; Tam et al., 1983). Idler and his associates (1983) have already succeeded in isolating two different gonadotropin fractions from flounders -- one associated with gonad maturation, the other with

vitellogenesis. Also, the presence of cells in the PI that are associated with reproductive hormones is not unique to platyfish or even other teleosts (e.g. ir-GTH in salmon, Ekengren et al., 1978). Ir-LHRH has also been localized in the PI cells of rats (Li et al., 1984).

In early maturering platyfish, ir-LHRH neurons appear in the NOR at 5 weeks and the number of ir-GTH vCPD cells has increased and continues to increase three to five fold over a 14 week period between five and eighteen weeks of age (sexual maturity). Late maturing sibs follow a pattern of development that begins later; ir-LHRH neurons appear in the NOR at nine to eleven weeks. Their appearance is associated with a proliferation of gonadotrops which occurs over a forty-nine week period (between ten to fifty-nine weeks of age), and ends with the presence of fewer ir-cells in the brain and pituitary than in early maturers. Plate 10 illustrates the correlation between the number of ir-LHRH containing neurons in the brain and the number of ir-GTH CPD and PI cells in the pituitary of both early and late maturing platyfish from one week old to adulthood.

The fact that the number of ir-LHRH containing perikarya in the NOR of both genotypes reaches a maximum value early in development (stage 1) at a time when none is seen in other regions of the brain and when ir-GTH and -LHRH are seen in certain pituitary cells suggests that the NOR participates in the early differentiation and function of gonadotrops in the vCPD and PI. By comparison the initial appearance of ir-LHRH perikarya in the NPP (both genotypes) and NLT (fibers only in late maturers) occurs at times (stage 2) related to the dramatic proliferation of gonadotrops in the CPD. It should be noted that late maturing animals, which never show ir-perikarya in the NLT, have considerably fewer CPD gonadotrops than early maturers. It would appear, therefore,

that the presence of ir-LHRH in perikarya of both the NLT and NPP is necessary for the maximum number of CPD gonadotrops to develop.

The lack of ir-LHRH in NLT perikarya of late maturers may be due to the fact that they are incapable of producing sufficient quantities of this neuropeptide to be detected by ICC methodology. Other possibilities should be considered. The presence of ir-fibers in the NLT of late maturers and the accumulation of ir-LHRH in NLT perikarya of 11-ketotestosterone injected $\underline{P}^2\underline{P}^5$ fish (Schreibman et al., 1983 and pg. 44) would preclude the suggestion that these neurons are incapable of LHRH production. It is also possible that the rates of NLT LHRH synthesis and release in late maturers are similar and, therefore, that LHRH does not accumulate in these neurons. Although cytological similarities between NLT perikarya of early and late maturers in stage 2 suggest that the dynamics of total protein synthesis and release are comparable in the two genotypes, the fact that only early maturers accumulate ir-LHRH in perikarya of the NLT indicates that some differences must exist between the genotypes with regard to NLT synthesis and/or release of LHRH in particular.

The portion of this study concerned with pituitary removal and hormone replacement further illustrates the dynamics of the interaction between LHRH and pituitary GTH. The results of ICC and RIA analyses demonstrate that hypophysectomy produces distinct changes in the distribution and quantity of LHRH in the brain. RIA results indicate that there is a decrease in LHRH content in the brains of hypophysectomized animals, which is not restored by the administration of salmon GTH. ICC indicates that while H animals show decreased ir-LHRH in the NLT and NPF, there is in fact an increase in NOR immunoreactivity.

Similarly, the administration of GTH also has a differential effect in different regions of the brain, that is, NOR perikarya and fibers show a marked decrease in the H induced increase in ir-LHRH, while NLT and NPP immunoreactivity is increased in fibers but not perikarya. On the whole, these results indicate a correlation between ICC and RIA determinations on the overall content of LHRH in the brain. However, they also illustrate that although RIA is a useful quantitative tool, it does not enable the detection of the ICC-demonstrable relative concentration changes in the distribution of the substance being measured. It is, therefore, much more enlightening if both techniques are employed in studies where organs are assayed.

ICC demonstrations of material which crossreacts with anti-mammalian LHRH in perikarya, fibers and pituitary cells in regions known to be associated with reproduction in the platyfish (Margolis-Kazan et al., 1981; Munz et al., 1981, 1982), and the fact that portions of the decapeptide are homologous in fish and mammals (Barnett et al., 1982; King and Millar, 1980), justify the use of mammalian LHRH antiserum in the RIAs in this study.

The different responses of the NOR, NPP and NLT to hypophysectomy and the fact that the times of appearance of ir-LHRH in the three centers between birth and puberty are different suggest that these brain areas differ in their roles in regulating BPG axis function. The region of the brain which first exhibits perikarya and fibers containing ir-LHRH is the NOR, and this occurs at a developmental stage that is characterized by low GTH levels due to the absence of the gonadotropic zone characteristic of sexually mature platyfish pituitary glands. Thus, it appears that the NOR functions when GTH levels are low, as, for example, in sexually immature platyfish and in mature fish following hypophysectomy. This

concept is further supported by the partial decrease of the H induced increase in NOR ir-LHRH in H GTH treated fish. In mammals too, brain nuclei containing the same type of hormone have been found to respond differently to hypophysectomy (Emanuele et al., 1981a,b; Hotstetter et al., 1981; Nallar and McCann, 1965; Souchek et al., 1981; Wenger et. al., 1978), thus extending the concept that regions of the brain that are associated with the regulation of a particular endocrine phenomenon may be affected differently by identical physiological signals. It has been suggested that, in rats, changes in the distribution of ir-LHRH material in the brain may be due to structural modifications which follow the surgical removal of the pituitary gland (Baker and Dermody, 1976). It has not been determined if any changes in the neuronal pathways of the NOR occur in hypophysectomized platyfish. However, the results from the ontogeny, and hypophysectomy and hormone replacement studies in platyfish lead to the speculation that shifts in LHRH content are physiologically based and not due to a remodeling of the brain cytoarchitecture. Also, these changes are presumably not related to the death of neurons, as has been reported in the supraoptic nucleus following hypophysectomy in rats (Raisman, 1973) since cell counts of random samples in this study indicated no differences between H and sham-operated fish in the number of neurons present in the NLT, NOR and NPP.

In removal of the pituitary gland has profound deleterious effects on the functioning of all endocrine organs and this complicates an analysis of the effects of hypophysectomy on specific physiological phenomena.

In fishes, little is known about the specifics of feedback mechanisms in the neuroendocrine system, and this is especially so for the effect of H on LHRH levels in the brain of fishes. Therefore, most

interpretations of the results in this study are, based on our understanding of endocrine control systems in other vertebrates, tempered by the knowledge that regulation of gonadotropic activity in teleosts is primarily neural, and not vascular (Peter and Nagahama, 1976; Peute et al., 1976). One would expect the removal of the pituitary gland, and the associated decrease in GTH, to lead to a depletion of stored LHRH in the brain as well as an increase in its synthesis. The cytological observations of high synthetic activity, along with the quantitative decrease in LHRH observed in the brain as determined by RIA and ICC, supports this interpretation, at least for the NPP and NLT. In the NOR, however, the evidence of high activity is associated with an increase in ICC-demonstrable ir-LHRH as well as in increase in basophilia and nuclear and nucleolar size, thus suggesting that in this brain region there is activated synthesis without increased, or even perhaps with decreased, LHRH release. The failure of GTH administration to restore LHRH to normal levels appears to conflict with interpretations based on BPG regulation in mammals; however, the quantity of GTH administered in this study may have been insufficient to reverse the effect of hypophysectomy on the LHRH stores. This explanation is supported by the fact that five 10-ug GTH injections on alternate days does lead to a partial return of normal cytology and ICC-demonstrable LHRH levels in the NOR, NPP and NLT. Experiments should, therefore, be conducted in which GTH is administered in greater quantities and/or for longer durations. If these proposed experiments result in a restoration of ICC- and RIA-detectable LHRH to levels characteristic of intact fish, they would support the concept that, in platyfish, pituitary gonadotropin acts in a shortloop feedback system to assist in the control of LHRH synthesis and release. On the other hand, the failure of GTH to restore LHRH levels would suggest that

the primary control, or one acting in conjunction with the shortloop mechanism, is dependent upon the direct contact of LHRH-conveying neuronal fibers with pituitary gland gonadotrops. Although the failure of GTH to restore LHRH to normal levels may also have been influenced by the quantity of gonadal hormones present in the hypophysectomized fish, the study indicates that even 5 weeks after hypophysectomy the histology of the ovary remains normal, thus making it likely that changes in gonadal steroid levels were minimal for the duration of these experiments. However, the quantity of GTH administered appears to be sufficient to result in the depletion of mature yolky oocytes and the stimulation of oogenesis, a phenomenon observed in other teleosts as well (Sundararaj et al., 1972).

It is clear that gonodal steroids are important in affecting a delicate balance among components of the BPG axis from birth to adulthood in platyfish. Neonatal fish already contain steroid synthesizing enzymes (Schreibman et al., 1982b). Metamorphic changes in the anal fin indicate that circulating levels of sex steroids appear early and increase at specific periods associated with maturational changes (Grobstein, 1948; Schreibman et al., 1982b).

The use of tritiated sex steroids and autoradiography have demonstrated steroid concentrating centers in the teleost brain. Radioactive material has been identified in the telencephalon close to the NOR, the NPP and the NLT (Kim et al., 1979a, b). Also, steroids labeled with tritium or fluorescein have been localized in pituitary cells in the CPD and PI (Kim et al., 1979a, b; Schreibman et al., 1982c). These findings suggest that sex steroids may directly influence secretory activity by binding to cells in the pituitary and brain.

This is further substantiated by experiments in which the

administration of androgens to immature fish of early and late maturing genotypes induced precocious sexual development which was manifested by the accumulation of ir-LHRH in the brain, a proliferation of gonadotrops, and sperm production (Schreibman et al., 1983). This experiment also demonstrated that different steroids affect different brain centers, since the administration of testosterone (T) led to the accumulation of ir-LHRH in the NOR and NPP, whereas 11-keto testosterone (11-K) led to its accumulation in the NOR and NLT. Even animals of late maturing genotype that received 11-KT had ir-NLT perikarya, a phenomenon never observed in normal development. The fact that the administration of sex steroids can induce the maturation of all components of the BPG axis suggests that under normal conditions, puberty may be induced by a sudden increase in circulating levels of sex steroids. A positive feedback of steroids has also been demonstrated in immature salmonids where the implantation of testosterone into the NLT and pituitary dramatically increased the content of pituitary GTH and stimulated gametogenesis (Crim and Evans, 1980, 1983). Furthermore, in vitro studies in salmonids demonstrate that the administration of androgens in conjunction with LHRH produces an increase in the number of gonadotrops that is greater (Fahraeus-van Ree et al., 1983), than that found when LHRH is given alone (van den Hurk and van de Kant, 1975; Billard, 1978; Gielen et al., 1982; Crim and Evans, 1983; Farhaeus-van Ree, et al., 1983).

This study demonstrates that the sequential appearance of LHRH producing centers in the platyfish brain precedes and is essential for the completion of pituitary gonadotrop development and the subsequent maturation of the gonads. The steps in this pattern of development are similar in both genotypes, although in late maturers they are more protracted and occur in older fish. It is well established that the I

gene controls the age and size at which platyfish become sexually mature (Kallman and Schreibman, 1973; Kallman et al., 1973; Schreibman and Kallman, 1977; Kallman and Borkoski, 1978). A basic question that remains to be answered is how the language of the genome is translated into the neuroendocrine physiological action that leads to the development of the reproductive system. The findings presented in this study suggest a number of focal points for future research on the site of action of the P locus.

The NOR is one of several likely candidates for the location of the P gene "switch" for reproductive development. It is the first region in the brain to contain ir-LHRH and, therefore, the NOR could function in regulating the gonadotropic activity of the immature pituitary. Presumably this would be accomplished by direct innervation of the ICPD and PI. The most significant role of the NOR, however, may well be one of relaying a message to other brain centers signaling the beginning of the process of puberty. This is suggested by the fact that the NOR innervates the other two brain centers in addition to the pituitary and that there are marked changes in its activity which are directly related to the initiation of activity in the NPP, NLT and pituitary. In short, it appears that the "cascade" phenomenon may be initiated by the NOR. Lesioning experiments might help clarify the functional interrelationship of the three brain centers.

The NOR is unique compared to the other ir-LHRH containing brain regions of platyfish by virtue of its anatomical and physiological position in the neuroendocrine system. Its association with visual and olfactory receptors (Munz et al., 1981, 1982; Schreibman et al., 1983, 1984; Crapon de Caprona and Fritzsch, 1983) and with the pineal gland, places it in a prime position for receiving environmental cues, important

to reproduction, and converting them into endocrine action in the BPG axis. The positive correlation between the development of nasal epithelium and the stage of sexual maturation, not chronological age (Schreibman et. al., 1984) further demonstrate the importance of the link between NOR related sensory receptors and P gene action.

The system in which the NOR occupies an important pivotal position is not unique to platyfish. It appears analogous to the nervus terminalis in the guinea pig which is the first region to contain ir-LHRH during development and whose ir-LHRH cells and processes are often intermingled with fiber bundles of the olfactory nerve (Schwanzel-Fukuda and Silverman, 1980; Schwanzel-Fukuda, et al., 1981). In humans, there is a reproductive anomaly known as Kallmann's Syndrome which results from the failure of the olfactory system to develop normally. This condition is characterized by deficiencies in LHRH and GTH, the failure of the gonads to develop, anosmia (loss of sense of smell) and occasionally color blindness. The administration of LHRH results in the restoration of pituitary gonadotropic function (Soules and Hammond, 1980).

Another possibility is that the P gene has operational significance in the NPP. The observation that the NOR at stage 2 in late maturers is significantly larger than that of early maturers suggests that it may be working harder to override a "genome-induced block" to the "switch" which may be present in the NPP of late maturers. The NPP is part of the anteroventral preoptic region, an area strongly implicated as one of the sites for origination of gonadotropin releasing hormone (GnRH) in many teleosts and numerous studies have identified ir-LHRH perikarya in th NPP whose fibers travel to the NLT and/or pituitary stalk (Schreibman et al., 1979; Borg, et al., 1982; Peter, 1983; Kah et al., 1984). That a dual control system may reside in the NPP is suggested by reports that it is

also a center for GTH release inhibitory factor (GRIF) activity (Peter and Paulencu, 1980; Chang and Peter, 1983 a or b). It is believed that this inhibitor is the catecholamine, dopamine (Chang and Peter, 1983a, b). Therefore, it is quite possible that the P gene in platyfish is responsible for the regulation of this, and/or other inhibitors of LHRH action. Bao (1981) and Bao and Kallman (1982) suggest that an inhibitor of GnRH may be responsible for the failure of hybrids of Xiphophorus to reach sexual maturation.

In the "cascade" scheme the role of the NPP is to initiate activity in the NLT which, with the participation of the NOR and NPP, leads to the development of the CPD gonadotropic zone in the pituitary and subsequent gonadal maturation. This sequence of events in the NPP, NLT and pituitary occurs rapidly. The existence of an effective communicative relay system between these two brain nuclei (NPP and NLT) and the pituitary is further suggested by experiments in goldfish where injections of monosodium glutamate (MSG) cause degeneration in areas of the NPP, NLT and pituitary stalk (Kah et al., 1983).

The failure of ir-perikarya to appear in the NLT of late maturing fish from stage 2 to stage 6 may have considerable significance in the understanding of delayed maturation, especially that found in homozygous Nigra fish ($\underline{P}^5\underline{P}^5$, NN) which frequently do not reach sexual maturity until they are more than one year old (Kallman and Borkoski, 1978) or sterile Xiphophorus which never become mature (Bao and Kallman, 1982). The inability of Munn, et al. (1981) to identify ir-LHRH in perikarya of the NLT of "store-bought" platyfish, might very well have been due to the fact that late maturing genotypes or sterile hybrids were used in that study.

The NLT is in close proximity to the nucleus recessus lateralis

(NRL) and nucleus recessus posterioris (NRP) of the paraventricular organ (PVO) which may act as a neuromodulator of LHRH function via neurotransmitter activity. Also, processes from the PVO which innervate the NLT and more rostral portions of the diencephalon have been identified in teleosts (Ekengren, 1975; Terlou et al., 1979). Fluorophores of the neurotransmitters dopamine and serotonin, have been identified in the PVO of numerous teleosts by use of Falck-Hillarp methodology (Terlou et al., 1979) and, most recently, immunocytochemical methods have been used specifically to localize ir-serotonin (5HT) in the NRL and NRP of platyfish (Margolis-Kazan et al., 1983) and goldfish (Kah et al., 1983). It would be interesting to speculate that it is serotonin, which has been shown to modulate LHRH synthesis and release in mammals (Jennes et. al., 1981), acting at the level of the NLT, which regulates the last step in the cascade control of pituitary maturation. This hypothesis could be evaluated by studying differences in the distribution of ir-5HT in early and late developing genotypes at various stages of their maturational process.

The inability to demonstrate ir-LHRH in NLT perikarya of late maturers is the only striking brain difference noted between mature platfish of the two genotypes, and therein might lie a clue to delayed maturation and the expression of the P gene "switch". The possible reduced LHRH production by the perikarya of the NLT may be caused by the fact that the P gene expresses itself in a quantitative fashion. Late maturers also have fewer CPD gonadotrops, and thus smaller gonads, which may be a reflection of these findings. These observations are remarkably similar to the endocrine profile of hypogonadal (hpg) mice, whose deficiency in hypothalamic LHRH results in a reduction of pituitary gonadotropin, poorly developed gonadotrops and the failure of accessory

sexual tissue growth (Sustarsic and Wolfe, 1979; McDowell, et. al., 1982a,b). Furthermore, injections of synthetic LHRH or the implantation of LHRH producing brain tissue from normal donors into the third ventrical of these hpg mice reverses these abnormalities (Charlton et al., 1983; Gibson et. al., 1983).

It is also possible that the P gene operates in a quantitative manner at the level of the pituitary gland by regulating its sensitivity to LHRH. This could be accomplished through the genetic control of either the number of gonadotrops or the number of effective LHRH receptor sites on GTH producing cells. In fact, the term "P" gene was originated to suggest that its action was at the level of the pituitary (Kallman et al., 1973), and this was based on the observation that the abrupt development of the gonadotropic zone in the vCPD coincides with the initiation of puberty (stage 2).

This discussion clearly indicates that the site(s) and mechanism(s) of action of the P gene are highly complex phenomena and that they may involve many components of the nervous and endocrine systems. The results of this investigation suggest that the P gene may express itself, either directly or indirectly at all levels of the HPG axis. The parameters discussed were prompted by the findings of this study, however, it is clear that other possibilities, that go beyond the boundaries of this report, should also be considered.

The mechanisms controlling puberty have been extensively reviewed by Ojeda, et. al., (1983) who concludes that "the onset of puberty can be visualized as the culmination of a cascade of changes which develop in a synchronized manner during reproductive immaturity. While these phenomena may be subject to genetic control, their [illegible] occurrence and tangible interaction are of fundamental importance for puberty to occur

at a proper age". The platyfish, Xiphophorus maculatus, represents a perfect model for the investigation of these relationships between the genome and the development of neuroendocrine structure and function.

Summary:

This study utilized cytological, cytometric, immunocytochemical and radioimmunological methods to evalute the activity of LHRH producing regions of the brain in relation to the pituitary gland and gonad, (1) from birth to adulthood, in sibling platyfish genetically determined to reach sexual maturity at different ages, and (2) in adult platyfish which were hypophysectomized and received gonadotropin hormone replacement.

The results of this study indicate that there is a sequential development of the three ir-LHRH containing areas in the brain that is directly related to stage of sexual maturation and not chronological age. The NOR is the first region to contain ir-LHRH which then appears in the anteroventral portion of the NPP followed by the NLT pars posterioris. This anterior to posterior sequence of development which has been termed the "cascade effect" is essentially similar in both early and late maturing genotypes, except that in late maturers, specific steps take place at similar developmental stages but in older animals, and require more time to be completed, and ir-LHRH is never found in perikarya of the NLT. The delay in time creates significant differences in the cytometric, cytological, and immunological characteristics of the three brain regions in fish of the same age but different genotype.

The appearance of LHRH producing centers in the brain precedes, and is essential for the completion of pituitary gonadotrop development and the subsequent maturation of the gonads. A direct correlation was found between the number of ir-LHRH containing neurons in the brain and the number of ir-GTH CPD and PI cells in the pituitary of both early and late maturers from one week to adulthood. In adult early and late maturers the number of ir-cells are similar in the NOR and PI, but late-maturers have significantly fewer ir-cells in the NPP, NLT and CPD. In both

genotypes, ir-LHRH containing perikarya in the NOR reach maximum values before the appearance of ir-LHRH in the NPP and NLT which occurs concomitantly with the proliferation of gonadotrops in the CPD.

The dynamics of the interaction between LHRH and pituitary GTH are further illustrated by the effects of pituitary removal and hormone replacement. The ICC and RIA analysis results demonstrate that hypophysectomy produces distinct changes in the distribution and quantity of LHRH in the brain. RIA indicates that there is a decrease in total LHRH content in the brains of hypophysectomized animals, which is partially restored by the administration of salmon GTH. ICC indicates that while H animals show decreased ir-LHRH in the NLT and NPP, NOR immunoreactivity increases. Similarly, the administration of GTH also has a differential effect in different regions of the brain.

The different responses of the NOR, NPP and NLT to hypophysectomy and the sequential accumulation of ir-LHRH in these three centers between birth and puberty suggest that they differ in their roles in regulating BPG axis function. It appears that the NOR functions when GTH levels are low as, for example, in sexually immature platyfish and in mature fish following hypophysectomy. The NOR is also unique compared to the other ir-LHRH containing brain regions of platyfish by virtue of its association with visual and olfactory receptors and with the pineal gland.

The results of this study indicate that the site(s) and mechanism (s) of action of the P gene are highly complex phenomena and that the P gene may express itself, either directly or indirectly, at various levels of the nervous and endocrine systems.

Table 1

P alleles and Pigment Markers Used in this Study*

Stock	Pedigree	Sex Chromosome	P Allele	Pigment Pattern
Jamapa Jp	$163A^{60(D)}$	X-Sp^1	P^1	Spot-sided
Jamapa Jp	$163B^{56(A)}$ $163B^{54(A)}$	X-Dr(Sd)	P^1	Red dorsal fin (Spotted dorsal)
Jamapa Jp	$163B^{54}$ $163B^{54(C)}$	Y-Sr	P^2	Stripe-sided
Belize Bp**	4034,4033, 4148,4248, 3834,3913, 3871	X-N^1(CPo)	P^5	Nigra(Caudal peduncle orange black-banded)

* adapted from Kallman and Borkoski (1978).

Sp^1 - spot-sided patterns of different populations are caused by different allelic Sp factors (Kallman and Borkoski,1978).

N^1 - black-banded patterns of different populations are caused by different N factors (Kallman and Borkoski, 1978).

Bp** - This chromosome has been introduced through a series of backcrosses into the Jamapa stocks (Kallman and Borkoski, 1978).

Table 2

Genetic Crosses and Progeny Used in this Study

Cross I:

P_1	X-N X-Sp (x) X-Sp Y-Sr	
F_1	(1) X-Sp X-Sp	(3) X-Sp Y-Sr
	(2) X-Sp X-N	(4) X-N Y-Sr

Cross II:

P_1	X-N X-Sp (x) X-N Y-Sr	
F_1	(1) X-Sp X-N	(3) X-Sp Y-Sr
	(2) X-N X-N	(4) X-N Y-Sr

Cross III:

P_1	X-Sp X-Sp (x) X-Sp Y-Sr
F_1	(1) X-Sp X-Sp
	(2) X-Sp Y-Sr

Table 3

Correlation of Gonopodium Development to Stage of Testis Maturation*

Stage of Gonopodium Formation (According to Kallman and Schreibman 1973)	State of Testis Maturation (According to van den Hurk 1974)
Undifferentiated anal fin	Stage I: Testis consists of 2 lobes with spermatogonia around central stroma.
	Stage II: As in I but with first sign of intratesticular efferent duct formation.
Stage 1: At 3-5 weeks of age fin rays 3, 4 and 5 shorten in males.	Stage III: Testis enlarges due to proliferation of spermatogonial cysts.
Stage 1 to Stage2: 3-4-5 ray complex enlarges; increase in the number of segments in ray 3 from 9 to 22.	Stage IV: 2 or 3 layers of cysts that contain spermatocytes.
Stage 3 to 6: Gonopodium completes differentiation into rod-like structure with terminal hook, blade and "spines" (teeth).	Stage V: 6 or 7 layers of cysts some of which contain spermatids or sperm cells (sperm cells may be seen in gonopodium stage 2 in some early maturing genotypes); hydration of efferent duct tubules.
	Stage VI: Efferent ducts fully developed and many spermatozeugmata present.

* Schreibman et al., (1982).

Table 4

List of Progeny Used in this Study

Genotype	P Alleles	Age (wks)	Sex	Stage	No. of Animals
Sp/SpSr	P^1P^1, P^1P^2	1	M/F	0	7
M/MSr	P^1P^5, P^2P^5	1	M/F	0	7
Sp/SpSr	P^1P^2, P^1P^1	5	M/F	1	10
M/MSr	P^1P^5, P^2P^5	5	M/F	1	7
MSr	P^2P^5	11	M	1	3
M/MSr	P^1P^5, P^2P^5	13	M/F	1	3
M/MSr	P^1P^5, P^2P^5	18,25	M/F	1	7
Sp/SpSr	P^1P^1, P^1P^2	10-12	M/F	2	15
M/MSr	P^1P^5, P^2P^5	26,33	M/F	2	12
SpSr	P^1P^2	20	M	6	3
Sp/SpSr	P^1P^2, P^1P^1	34	M/F	6	10
M/MSr	P^1P^5, P^2P^5	59,73	M/F	6	7
Sp	P^1P^1	51	F	Sexually Mature (Hypophysectomy Study)	95

Table 5

Total Volume and Total Number of Neurons (Mean ± S.E.) for Both Brain Hemispheres

Pedigree	Genotype **	Age (wks)	Stage	No. of Animals	NSR V(mm^3)±	No. of Neurons*	NPP V(mm^3)±	No. of Neurons*	NLT V(mm^3)±	No. of Neurons*
1633,1656(A) 4034	Sp/SpSr	1	0	7	.01± .001	78± .05	.008± .002	56± .05	.01±.008	50.4±.06
4034	N/NSr	1	0	7	.01± .002	78± .08	.006±.001	56± .05	.01±.006	50.3±.09
1634,4033, 1633[1]	Sp/SpSr	5	1	10	.02± .006	78± .10	.009± .001	56± .50	.03±.004	50.6±.08
4033,4148	N/NSr	5	1	7	.02± .004	78±.08	.008± .001	56± .40	.02±.007	51± .07
4033	NSr	11	1	3	.02± .006	78±.05	.01 ± .001	56± .70	.03±.006	50.2±.03
4033	N/NSr	13	1	3	.02± .001	78±.10	.01 ± .006	56± .03	.03±.004	50.2±.02
4148,4033, 3873,3834	N/NSr	18,25	1	7	.02± .006	78±.05	.015± .002	56± .07	.03±.006	50.5± .04
1633[54],4033, 4148	Sp/SpSr	10-12	2	15	.025±.004	78±.10	.02 ± .001	56± .09	.05±.008	50.6±.05
4033,4148, 3873,3834	N/NSr	26,33	2	12	.03± .004	78±.07	.03 ± .001	57± .06	.03±.006	51± .09
4033	SpSr	20	6	3	.03± .002	78±.05	.05± .008	56± .50	.02±.006	50±.10
4033,4148, 1633[54](A),	Sp/SpSr	34	6	10	.03± .003	78±.06	.05± .006	57±.04	.02±.007	51±.10
4148,3873,	N/NSr	59	6	3	.04± .006	78±.03	.04± .008	57±.30	.02±.008	51±.07
4033	N/NSr	73	6	4	.04± .003	78±.02	.04± .003	57±.20	.02±.003	51±.10

* Corrected according to Abercrombie (1946)

** Sp/SpSr - early maturer , N/NSr , late maturer

- Volume in cubic millimeters

Table 6
Morphometric Analysis of NOF from One Week-Old to Adulthood

Pedigree	Genotype	No. of Animals	Age (wks)	Stage	Cell Index(um)*	Nuclear Index(um)*	Nuclear Area(um^2)*
1632[56(A)],4034	Sp/SpSr	7	1	0	4.4 ± .10	3.5 ± .08	8.1 ±.20
4034	N/NSr	7	1	0	4.4 ± .08	3.4 ± .07	8.0 ±.10
163A, 4033,163B	Sp/SpSr	10	5	1	5.3 ± .03	4.5 ± .08	14.1 ±.20
4033,4148	N/NSr	7	5	1	4.5 ± .12	4.0 ± .09	11.1 ±.60
4033	NSr	3	11	1	5.9 ± .03	5.0 ± .03	17.2 ±.30
4033	N/NSr	3	13	1	6.3 ± .05	5.3 ± .10	18.8 ±.10
4148,4033,3913, 4034	N/NSr	7	18,25	1	7.0 ± .20	5.6 ± .04	22.1 ± .03
1632[54],4033,4148	Sp /SpSr	15	10-12	2	6.3 ± .09	5.2 ± .07	17.8 ±.03
4033,4148,3913, 4034	N/NSr	12	26,33	2	6.8 ± .10	5.5 ± .09	22.1 ± .60
4033	SpSr	3	20	6	6.6 ± .03	5.5 ± .04	18.9 ±.10
4148,4033,1632[54]	Sp/SpSr	10	34	6	6.5 ± .10	5.5 ± .03	18.9 ±.14
4148,3873,3913	N/NSr	3	59	6	7.0 ± .05	5.6 ± .10	22.3 ±.29
4033	N/NSr	4	73	6	7.0 ± .04	5.6 ± .20	22.3 ±.29

* Mean ± S.E.

Table 7

Morphometric Analysis of NPP from Birth to Adulthood

Pedigree	Genotype	No. of Animals	Age (wks)	stage	Cell Index(um)*	Nuclear Index(um)*	Nuclear Area(um^2)*
1631[56(A)],4034	Sp/SpSr	7	1	0	3.4 ± .20	3.1 ± .10	7.2 ± .40
4034	N/NSr	7	1	0	3.6 ± .20	2.8 ± .20	6.8 ± .50
1631[56(1)],4033	Sp/SpSr	10	5	1	4.1 ± .03	3.6 ± .05	8.8 ± .10
4031,4148	N/NSr	7	5	1	3.6 ± .05	3.2 ± .01	7.2 ± .04
4033	NSr	3	11	1	3.6 ± .10	3.1 ± .09	6.8 ± .05
4033	N/NSr	3	13	1	4.3 ± .30	3.6 ± .10	8.3 ± .40
3913,4033,3913, 3834	N/NSr	7	18,25	1	4.3 ± .06	3.5 ± .10	9.2 ± .30
1631[54],4033, 3910	Sp/SpSr	15	10-12	2	4.7 ± .07	4.5 ± .06	13.9 ±.30
4033,4148,3913, 3834	N/NSr	12	26,33	2	4.4 ± .01	4.4 ± .02	12.4 ±.20
4033	SpSr	3	20	6	5.5 ± .10	5.3 ± .03	23.0 ±.20
4033,4248, 1631[54(A)]	Sp/SpSr	10	34	6	5.5 ± .21	5.3 ± .03	23 ± .20
4148,3873, 3913	N/NSr	3	59	6	5.4 ± .10	5.3 ± .02	22.8 ±.10
4033	N/NSr	4	73	6	5.4 ± .12	5.3 ± .02	22.8 ± .12

* m ± S.E.

Table 8

Morphometric Analysis of NMT from One Week-old to Adulthood

Pedigree	Genotype	No. of Animals	Age(wks)	Stage	Cell Index(um)*	Nuclear Index(um)*	Nuclear Area(um^2)*
4034,1688$^{56(A)}$	Sp/SpSr	7	1	0	2.9 ± .05	2.6 ± .09	5.3 ± .30
4034	N/NSr	7	1	0	2.8 ± .06	2.5 ± .10	4.6 ± .03
1688$^{56(A)}$,4033	Sp/SpSr	10	5	1	3.4 ± .04	3.0 ± .03	6.3 ± .08
4033, 4148	N/NSr	7	5	1	2.9 ± .03	2.2 ± .06	5.2 ± .10
4033	NSr	3	11	1	3.1 ± .07	2.7 ± .06	5.8 ± .30
4033	N/NSr	3	13	1	3.1 ± .06	2.7 ± .06	5.5 ± .30
148,4033,3913, 3934	N/NSr	7	18,25	1	3.3 ± .03	2.9 ± .20	6.1 ± .08
1688$^{56(A)}$,4033, 4148	Sp/SpSr	15	10-12	2	4.0 ± .06	3.6 ± .05	8.8 ± .50
4033,4148,3913, 3934	N/NSr	12	26,33	2	3.5 ±.03	3.2 ± .03	7.9 ± .10
4033	SpSr	3	30	6	3.7 ± .07	3.5 ± .05	7.4 ± .50
4148,4033, 1688$^{56(A)}$	Sp/SpSr	10	34	6	3.7 ± .07	3.5 ± .02	7.4 ± .51
4148,3873, 4034	N/NSr	3	59	6	3.1 ± .04	3.0 ± .05	7.2 ± .05
4033	N/NSr	4	73	6	3.1 ± .06	3.0 ± .03	7.2 ± .04

Table 9

Correlation Between Number of Ir-LHRH Containing Perikarya in Brain and Number of Ir-LHRH and Ir-BGTH Containing Cells in Pituitary

Genotype	Age (wks)	Stage	#L-CPD Ir-Cells*	#V-CPD Ir-Cells*	#PI Ir-Cells*	#NOR Ir-Peri.*	#NPP Ir-Peri.*	#MLT Ir-Peri.*
Sp/SpSr	1	0	15.2 ±.1	3.0±.01	8.0±.2	0	0	0
+/+Sr	1	0	13.8 ±.5	2.5±.3	6.8±.2	0	0	0
Sp/SpSr	5	1	30.6 ±.1	8.0±.04	9.8±.9	80	0	0
+/+Sr	5	1	17.2 ±.7	3.0±.5	7.8±.2	0	0	0
+Sr	11	1	28.0 ±.9	12.7±1	19.1±.1	79	0	0
+/+Sr	13	1	31.3 ±1.3	17.3±1.3	19.1±.1	78	0	0
+/+Sr	18,25	1	42.5 ±.5	27.5±.1	22.5±.2	78	0	0
Sp/SpSr	10-12	2	59.1 ±.3	101.5±5	24.5±.4	78	25	45
+/+Sr	26,33	2	49.6 ±.5	80.1±3	23.0±.1	76	23	0
SpSr	20	6	77.6 ±.1	131.9±3	30.7±1.0	79	50	15
Sp/SpSr	34	6	78.3 ±2.1	134.5±4	30.6±2	79	52	17
+/+Sr	59	6	64.1 ±1.4	104±10.2	28.8±1.8	78	35	0
+/+Sr	73	6	65.4 ±2.4	103± 5.2	28.8±2	78	35	0

* Mean ± S.E.
Peri. = Perikarya

Table 10

Morphometry of Gonadotropic Cells from One Week-old to Adulthood

Pedigree	Genotype	No. of Animals	Age(wks)	LCED C.I. (um)*	LCED N.I. (um)*	vCED C.I. (um)*	vCED N.I. (um)*
4034,2165$^{56(4)}$	Sp/SpSr	7	1	3.5±.03	2.5±.05	3.1±.02	2.1±.10
4034	N/NSr	7	1	3.5±.01	2.5±.03	3.1±.03	2.2±.08
2165$^{56(4)}$,4033 1033^{54}	Sp/SpSr	10	5	3.8±.02	2.9±.02	3.2±.03	2.5±.03
4033,4148	N/NSr	7	5	3.1±.02	2.6±.05	3.0±.07	2.1±.07
4033	NSr	3	11	3.4±.06	2.7±.01	2.9±.06	2.4±.10
4033	N/NSr	3	13	3.7±.06	2.7±.06	3.3±.10	2.3±.10
4148,4033, 3913,3834	N/NSr	7	18,25	4.0±.04	2.9±.07	3.4±.10	2.3±.10
1033^{54},4033	Sp/SpSr	15	10-12	4.2±.01	3.0±.02	4.0±.07	3.0±.02
4033.4148, 3913,3834	N/NSr	12	26,33	3.9±.20	2.7±.05	3.8±.30	2.7±.05
4033	SpSr	3	20	4.4±.60	3.0±.03	4.3±.05	3.0±.06
4033,4148, 2165$^{56(4)}$	Sp/SpSr	10	34	4.4±.42	3.0±.06	4.3±.03	3.0±.06
4148,3834	N/NSr	3	59	4.2±.08	3.0±.05	4.0±.02	2.9±.06
4033	N/NSr	3	73	4.2±.10	3.0±.04	3.9±.10	2.8±.02

Mean ± S.E.

Table II

Morphometric Analysis of Pars Intermedia Cells From One Week-Old to Adulthood

Pedigree	Genotype	Stage	No. of Animals	Age(wks)	Cell Index (um)*	Nuclear Index (um)*
[illegible]	Sp/SpSr	0	7	1	3.1±.60	2.1±.06
[illegible]	N/NSr	0	7	1	3.0±.40	2.0±.04
[illegible]	Sp/SpSr	1	10	5	3.4±.50	2.7±.05
[illegible]	N/NSr	1	7	5	2.9±.60	2.6±.06
[illegible]	NSr	1	3	11	4.8±.08	3.0±.04
[illegible]	N/NSr	1	3	13	5.0±.06	2.9±.10
[illegible]	N/NSr	1	7	18,25	5.1±.08	3.0±.08
[illegible]	Sp/SpSr	2	15	10-12	5.2±.08	2.8±.20
[illegible]	SpSr	6	3	20	5.0±.20	2.9±.06
[illegible]	N/NSr	2	12	26.33	4.4±.50	2.6±.26
[illegible]	Sp/SpSr	6	10	34	4.9±.10	2.9±.20
[illegible]	N/NSr	6	3	59	5.5±.60	2.8±.04
[illegible]	N/NSr	6	4	73	5.5±.50	2.8±.03

Table 12

Morphometry (Mean ± S.E.) of Sexually Mature Fish Hypophysectomized (H) for 1,3, and 5 Weeks and Injected with SG-G100

Pedigree	No. of Fish	Experimental Condition	NCR C.I. (um)	NCR N.I. (um)	NPP C.I. (um)	NPP N.I. (um)	NLT C.I. (um)	NLT N.I. (um)	RIA pg/brain
163A	9	Sham-operated 1,3,5 weeks	6.8±.1	6.0±.05	6.4±.1	5.0±.1	4.0±.05	3.5±.03	----
163A	25	Sham-operated saline-injected	6.8±.06	6.0±.1	6.3±.2	5.0±.1	4.0±.1	3.5±.05	649±28 (n=8)
163A	7	H - 1 week	6.8±.1	6.1±.1	6.1±.1	5.0±.1	4.3±.03	3.6±.02	----
163A	7	H - 3 weeks	7.1±.1	6.7±.3	6.6±.2	5.4±.2	4.6±.05	4.0±.05	----
163A	7	H - 5 weeks	7.4±.02	6.6±.1	6.7±.3	5.6±.5	5.0±.6	4.0±.02	----
163A	25	H + saline-injected	7.4 ±.1	6.5±.1	6.7±.4	5.6±.5	5.0±.6	4.0±.04	446±31 (n=12)
163A	25	H + SG-G100	6.8±.2	6.3±.01	6.2±.01	5.1±.02	4.5±.1	3.6±.07	362±36 (n=11)

pg/brain - picograms per brain

The pituitaries of the 8 Sham-operated fish were pooled and gave a value of 507.5 picograms of ir-LHRH in the assay.

C.I. = Cell Index ; N.I. = Nuclear Index

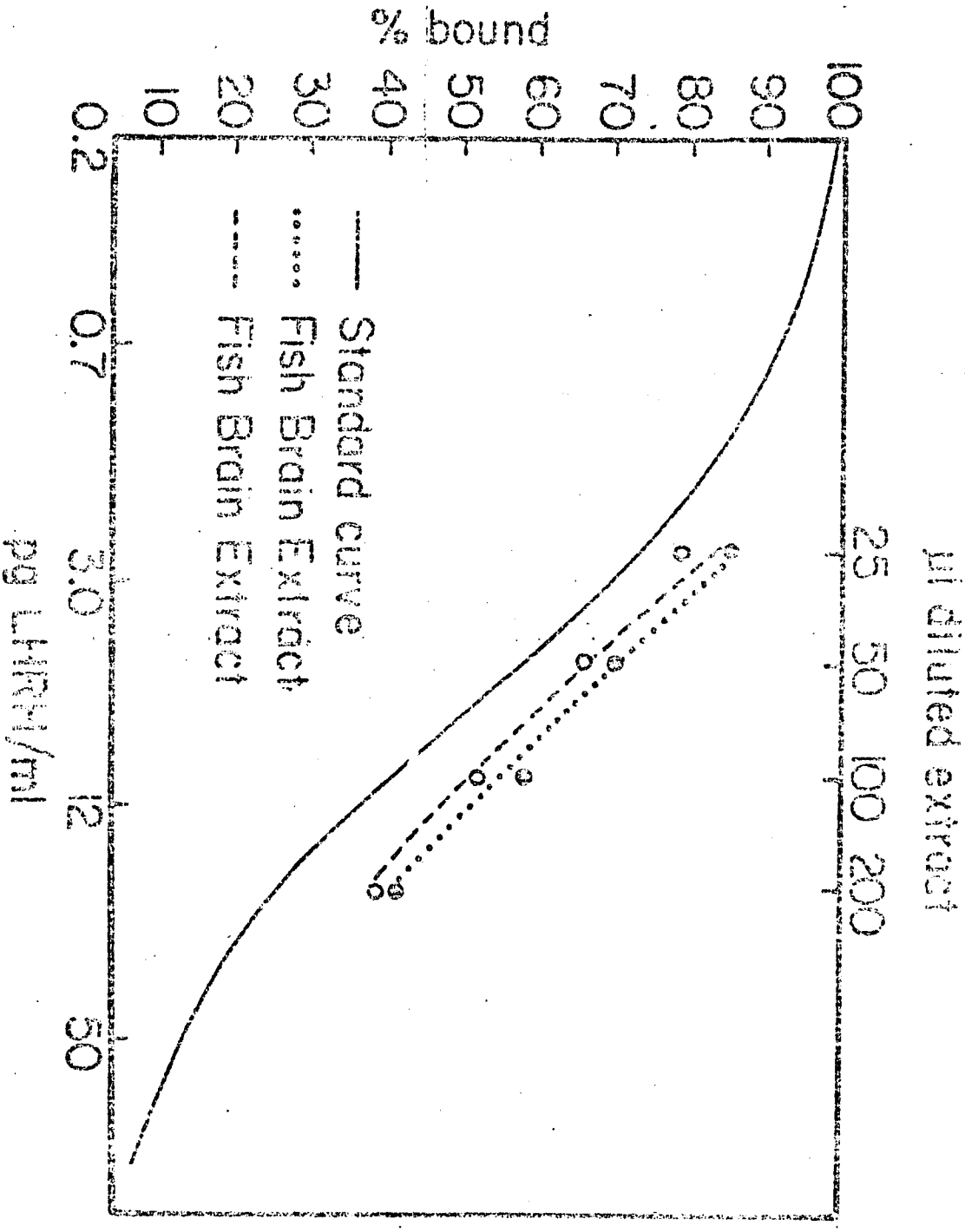


Plate 1. Radioimmunoassay of fish brain LHRH. Relationship is shown bwteen standard curve and both samples of fish brain extract.

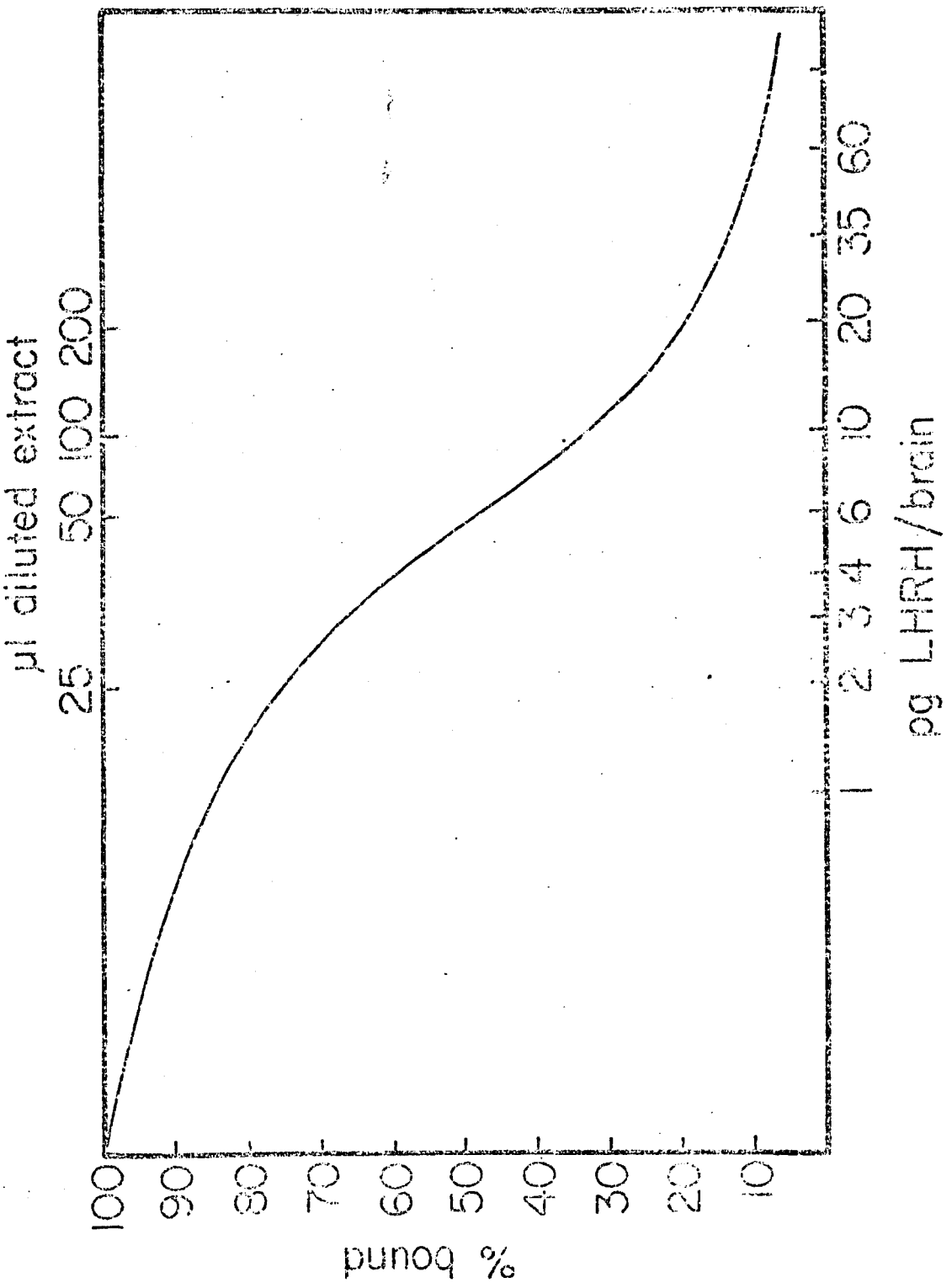


Plate 2. Radioimmunoassay of fish brain LHRH. Actual curve used to evaluate the effect of hypophysectomized ir-LHRH levels. Interassay value equals 1.791 picograms per brain; Intra-assay value equals 0.992 picograms per brain.

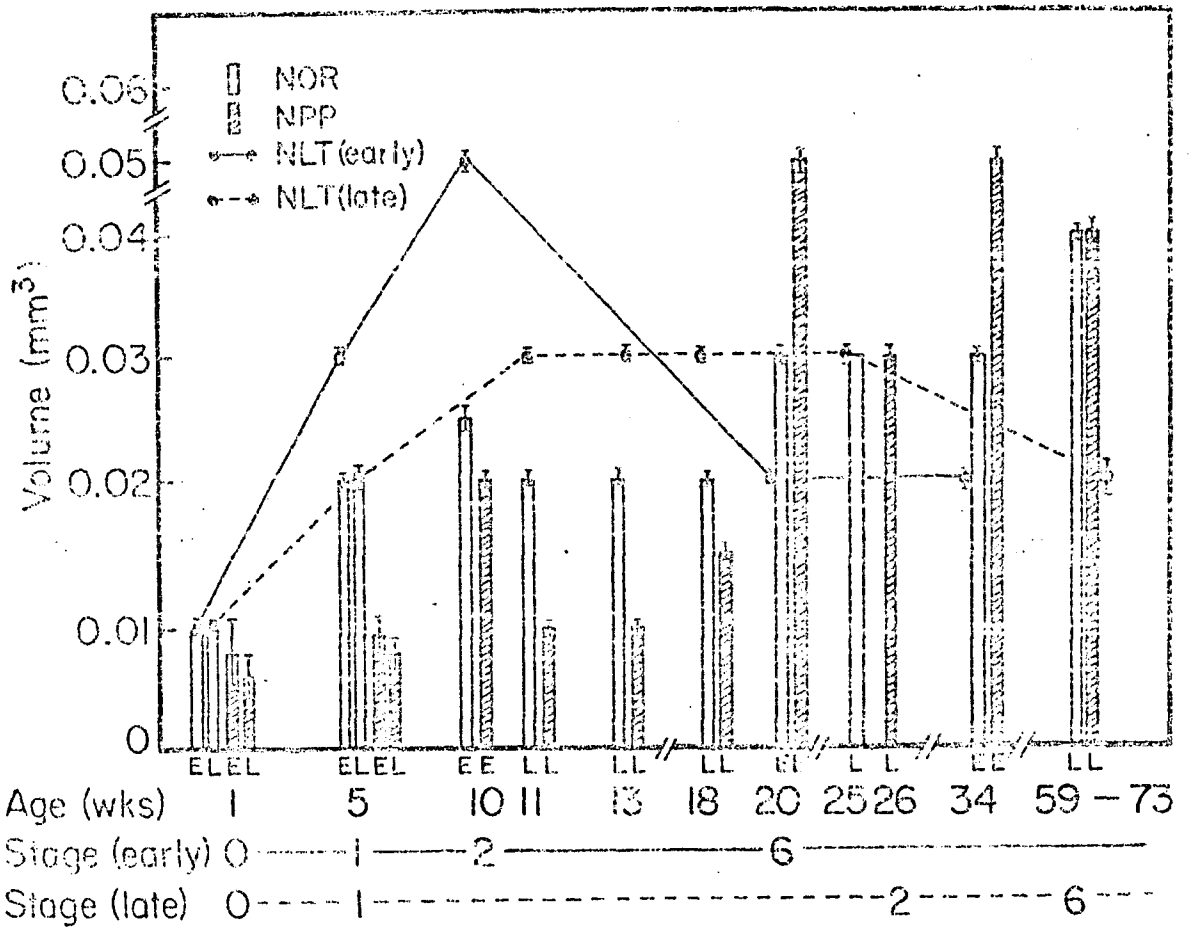


Plate 3. Total volume of the NOR, NPP and NLT in early and late maturers; E, for early; L for late. NOR and NPP represented by bars; NLT , represented by lines (for same stages and ages of development).

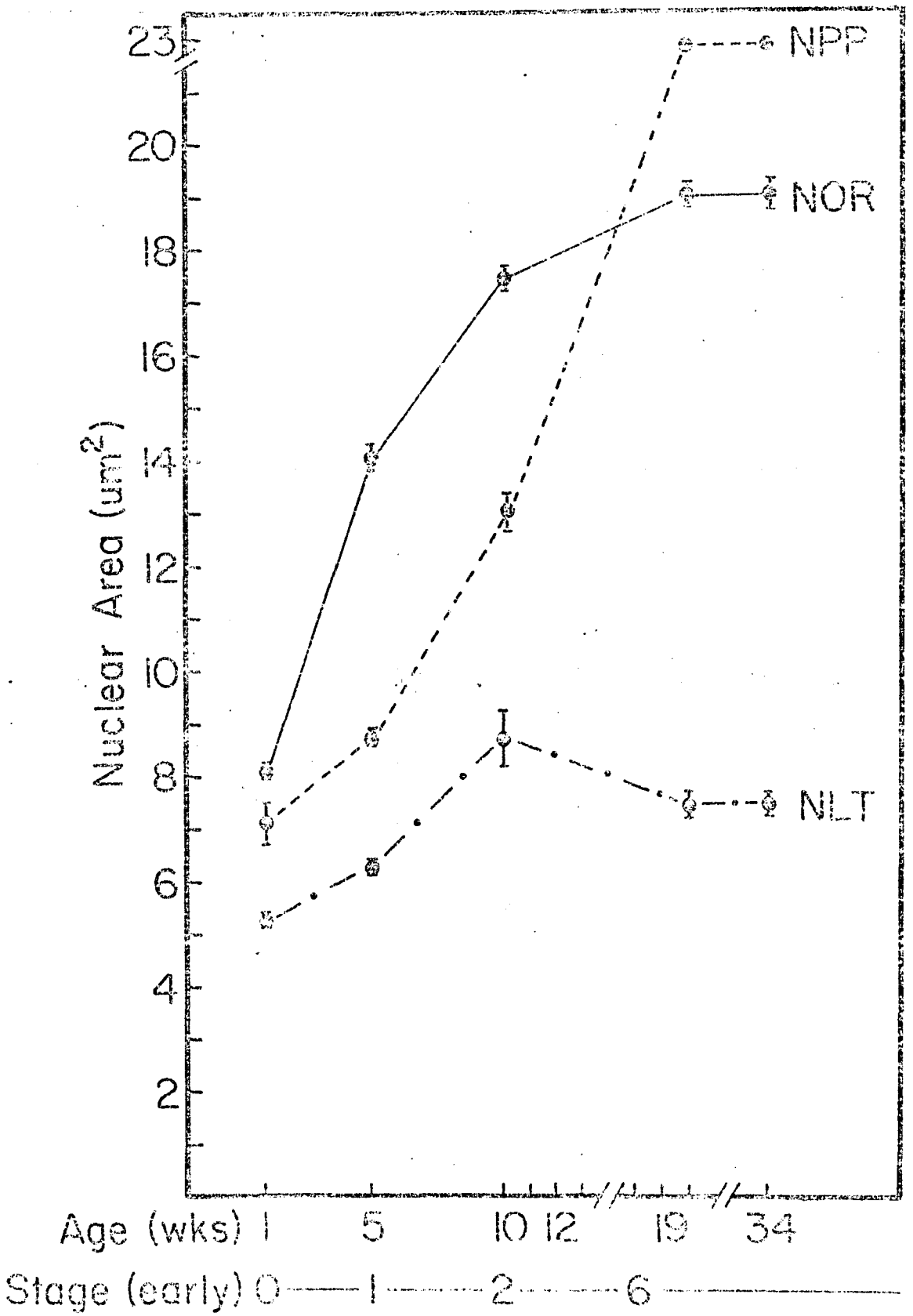


Plate 4. Nuclear area of the NOR, NPP and NLT in early maturers.

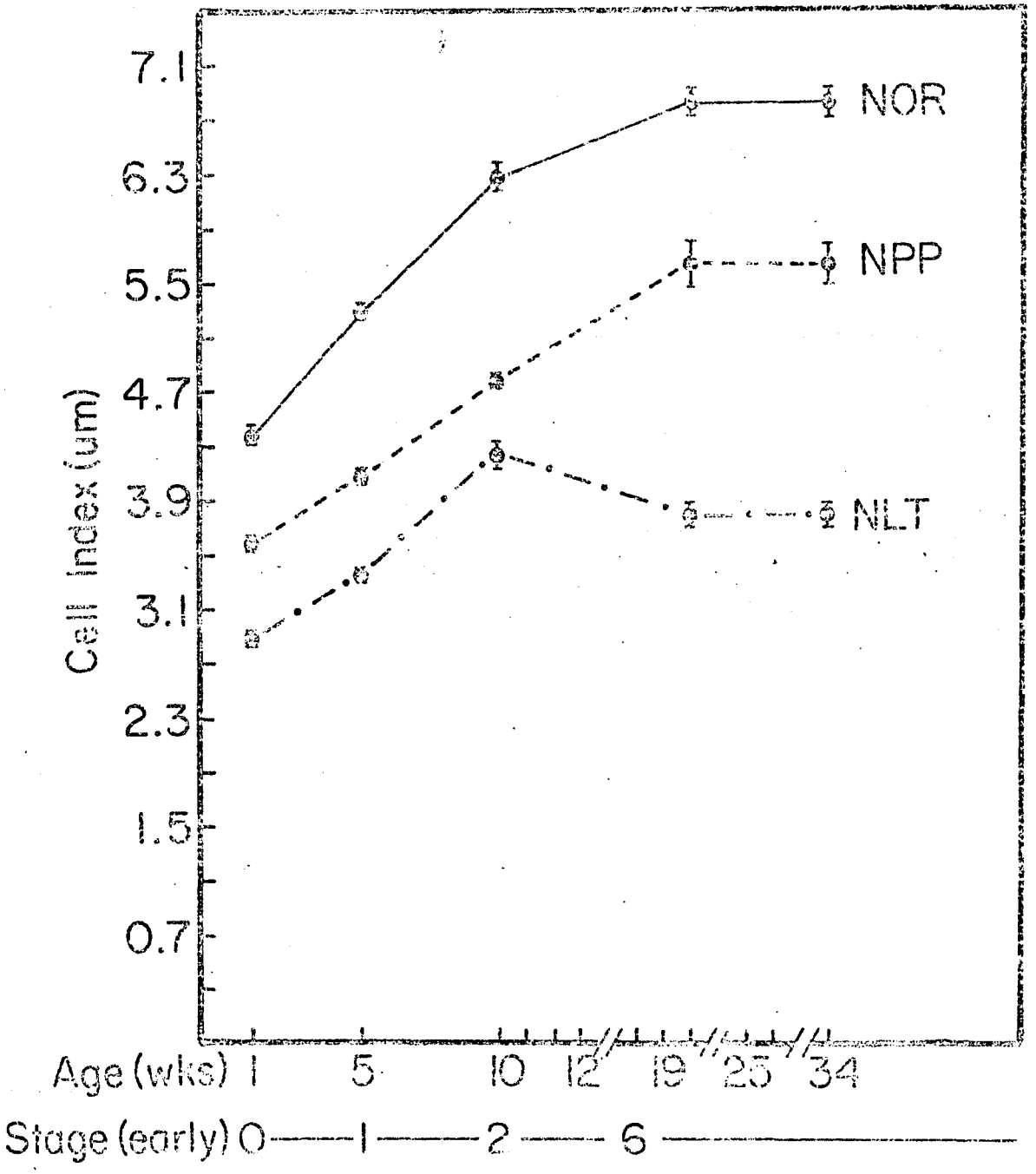


Plate 5. Cellular index of the NOR,NPP and NLT in early maturers.

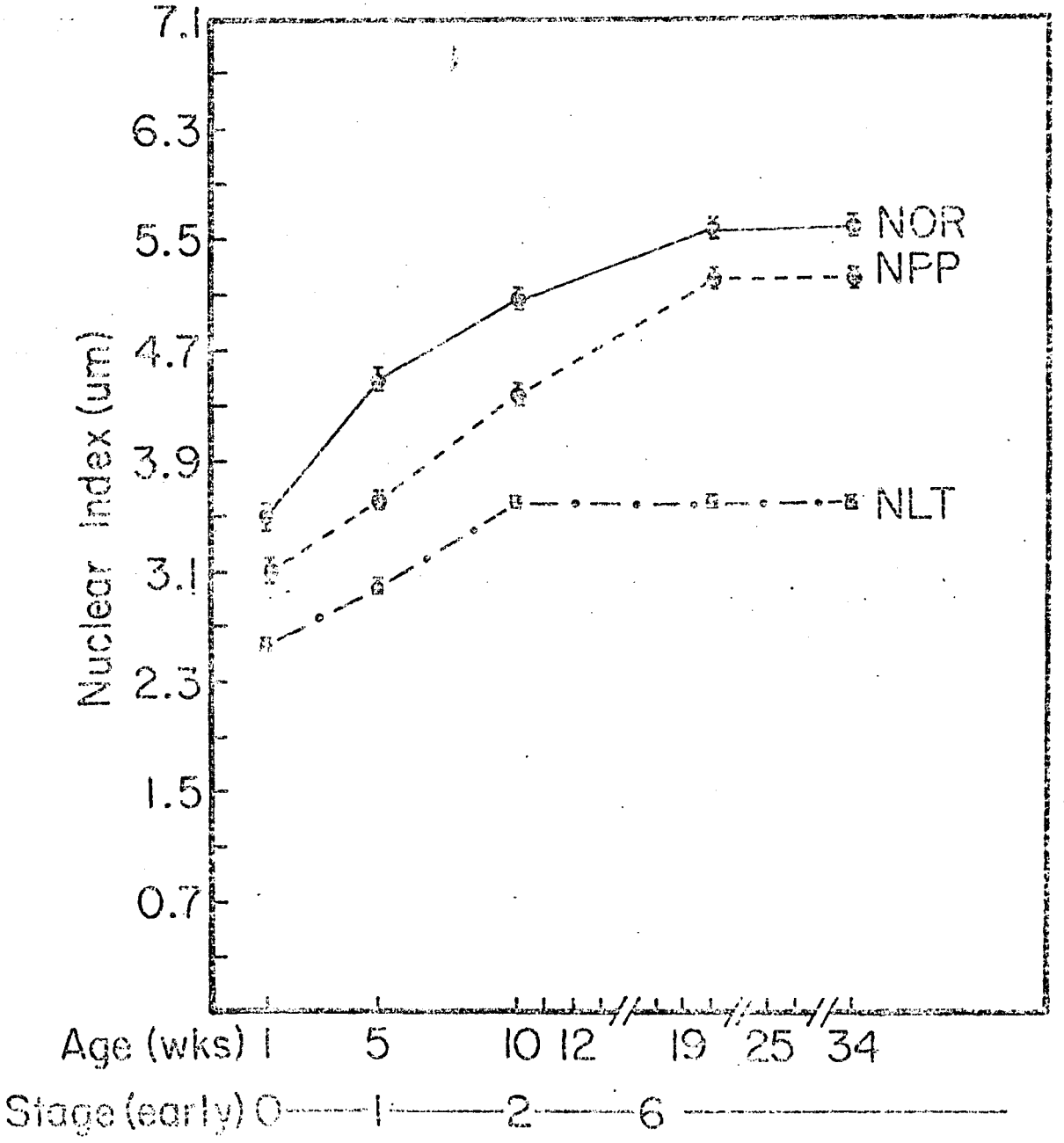


Plate 6. Nuclear index of the NOR,NPP and NLT in early maturers.

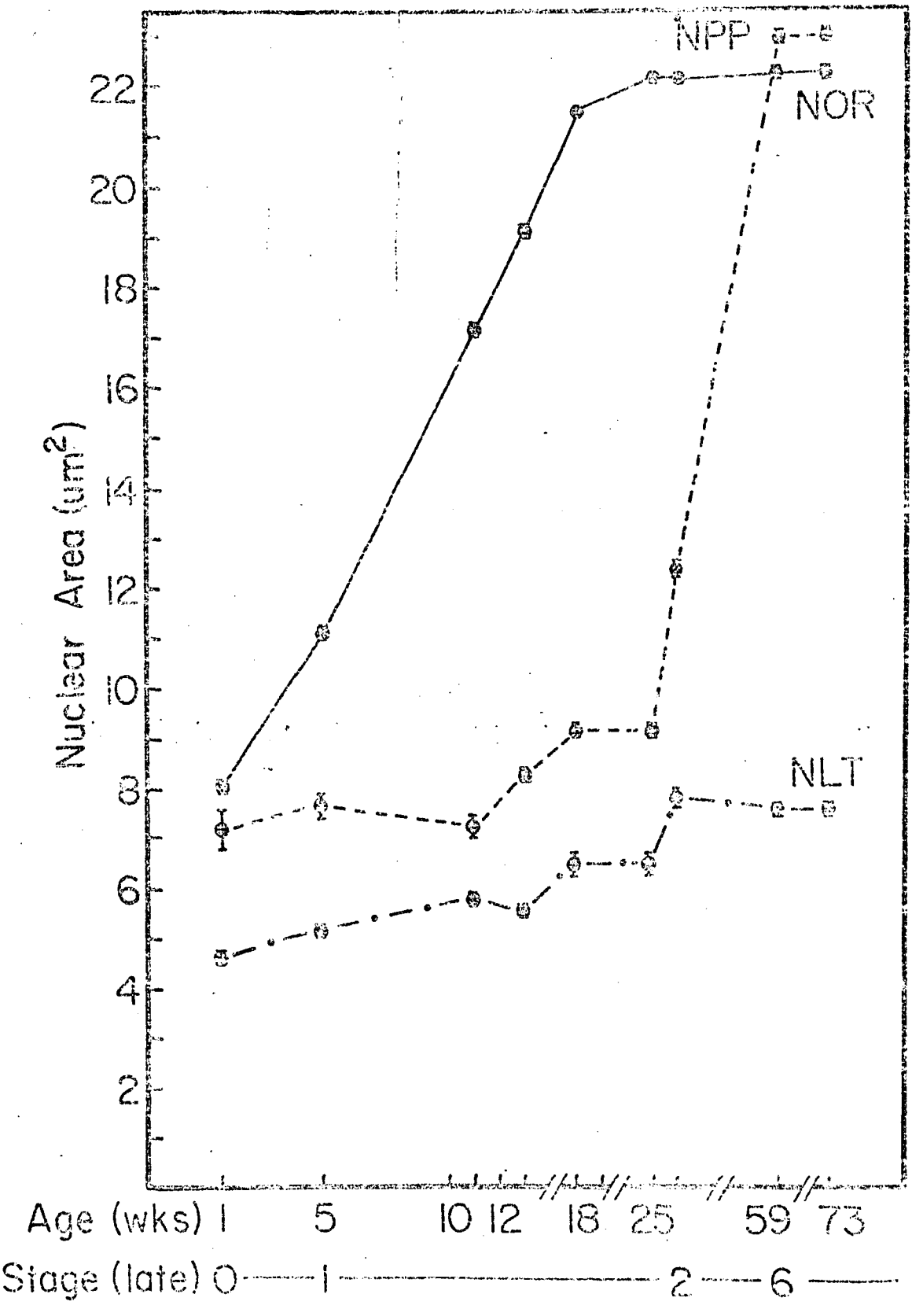


Plate 7. Nuclear area of the NOR,NPP and NLT in late maturers.

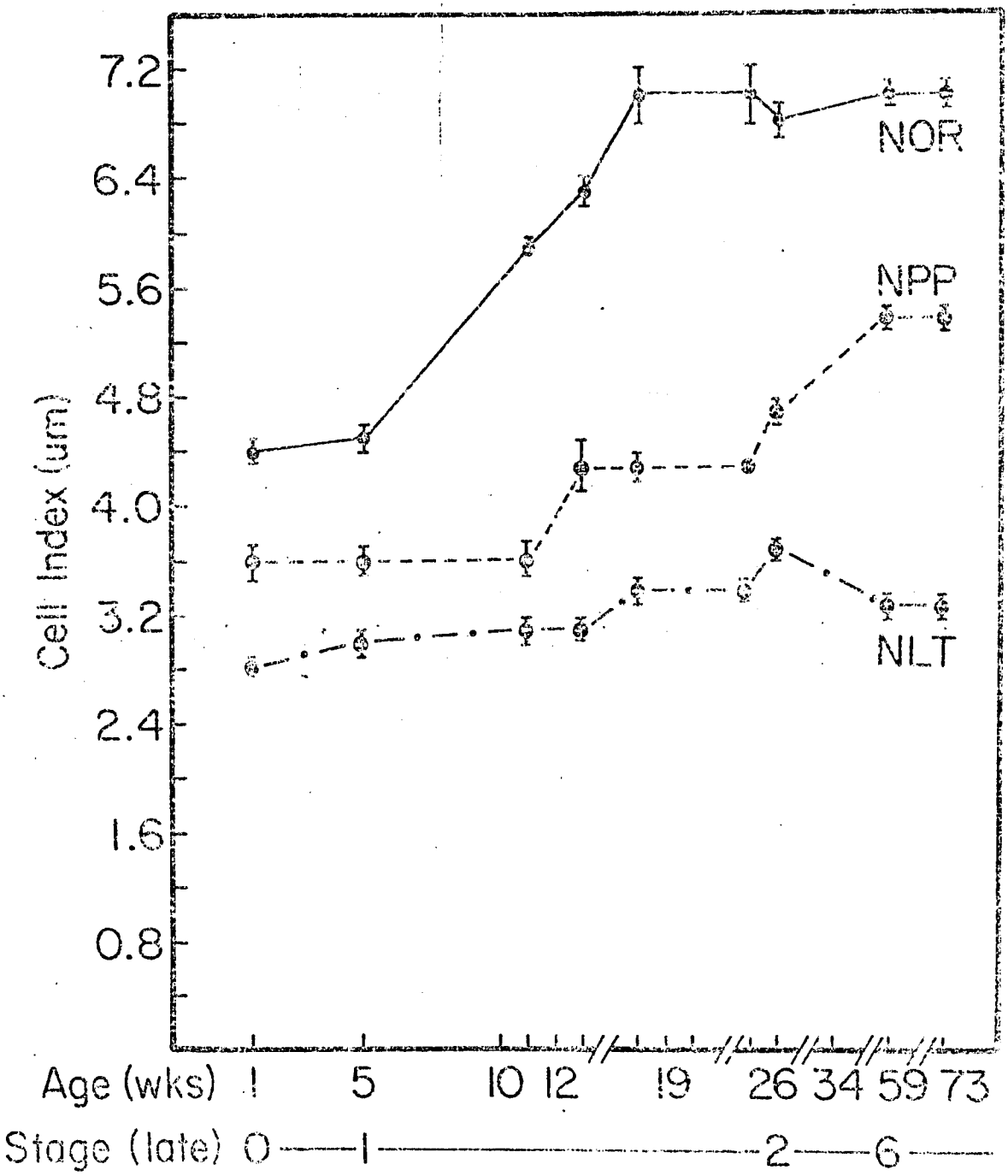


Plate 8. Cellular index of the NOR,NPP and NLT in late maturers.

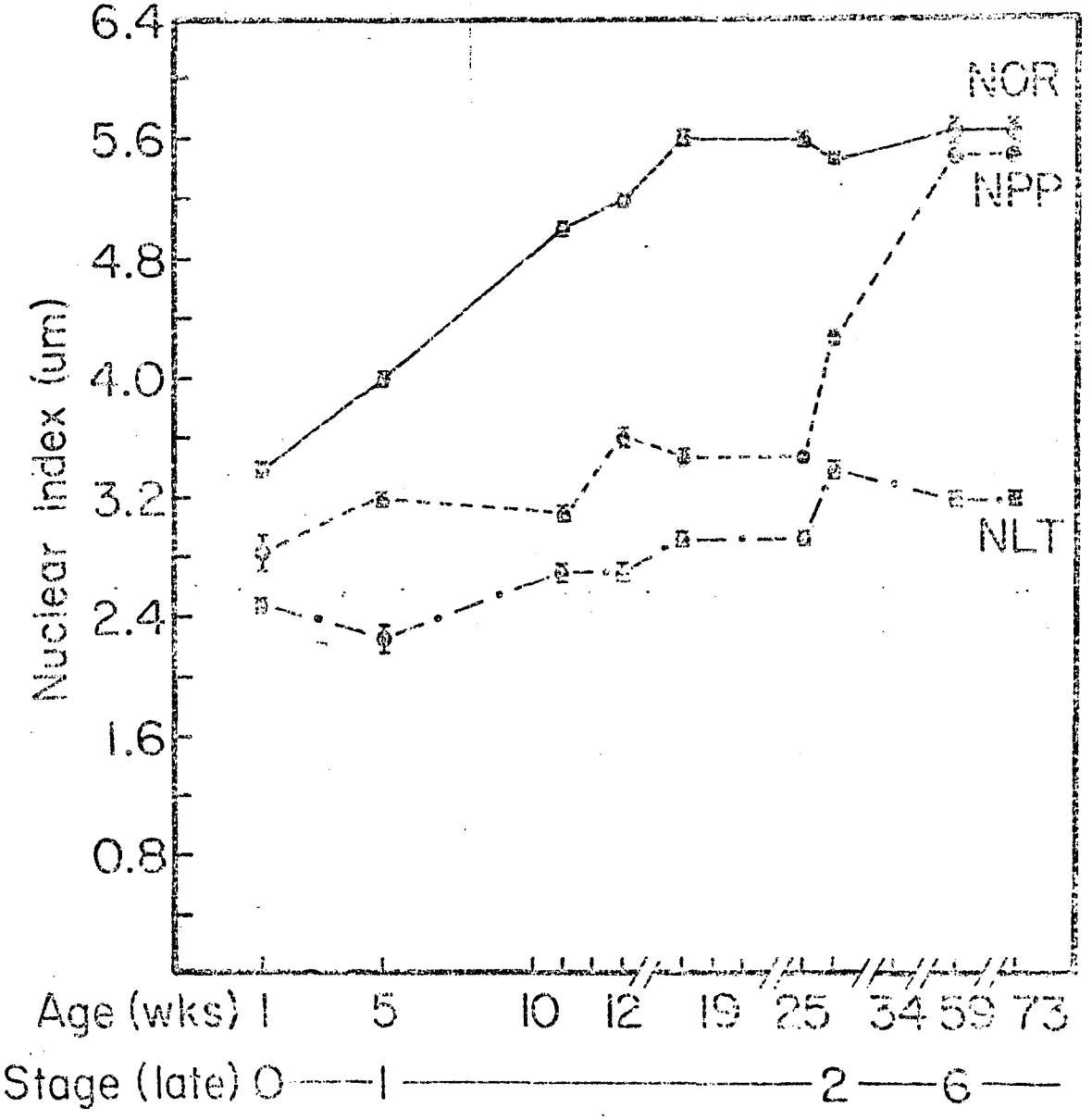


Plate 9. Nuclear index of the NOR,NPP and NLT in late maturers.

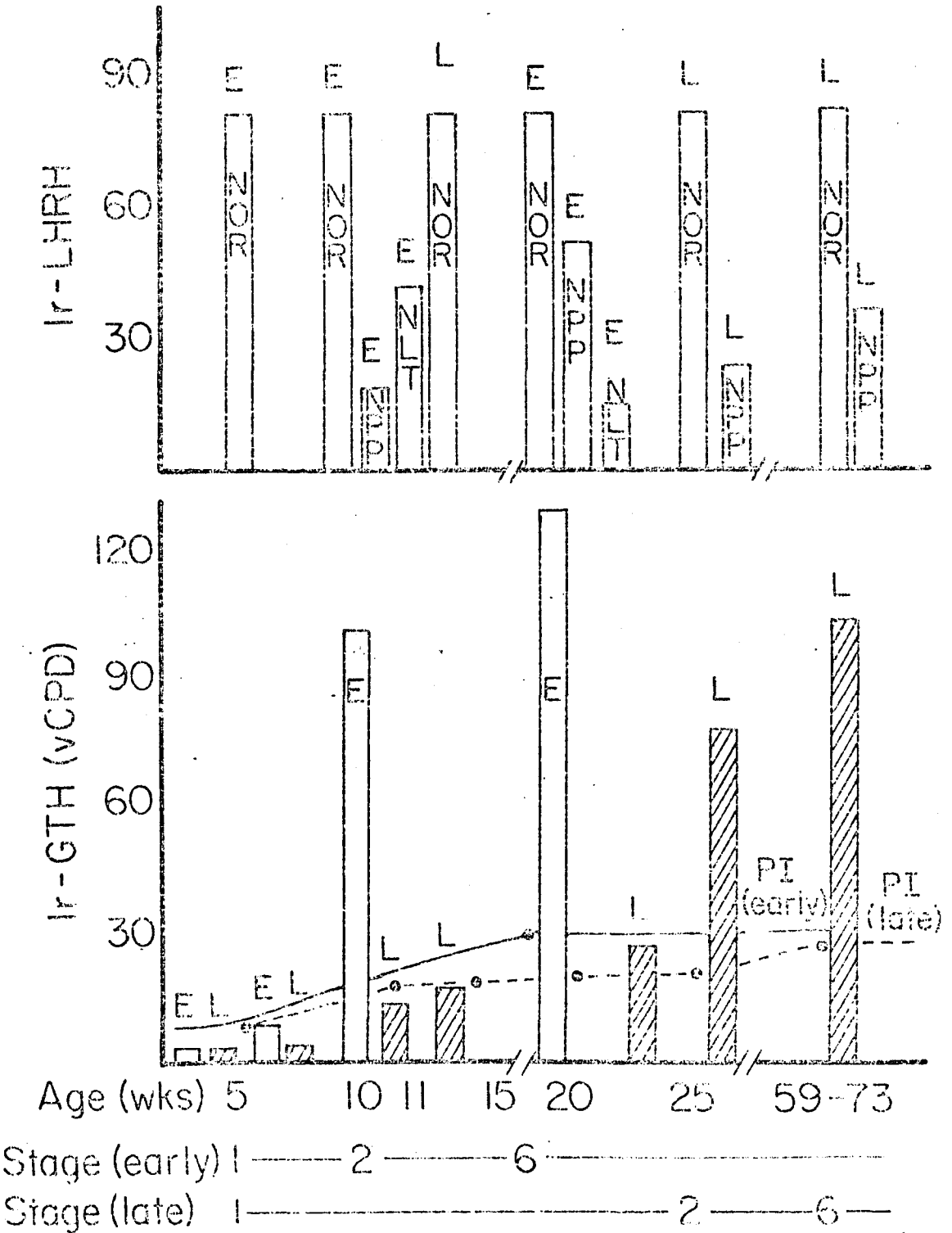


Plate 10. Upper panel; Number of ir-LHRH containing perikarya in the NOR, NPP and NLT of early (E) and late (L) maturers. Bottom panel: Number of ir-GTH containing cells in the vCPD of early (open bars) and late (cross-hatched bars) maturers. Drawn lines on this graph represent number of ir-GTH cells in PI. Age and stage scales used for both upper and lower panels.

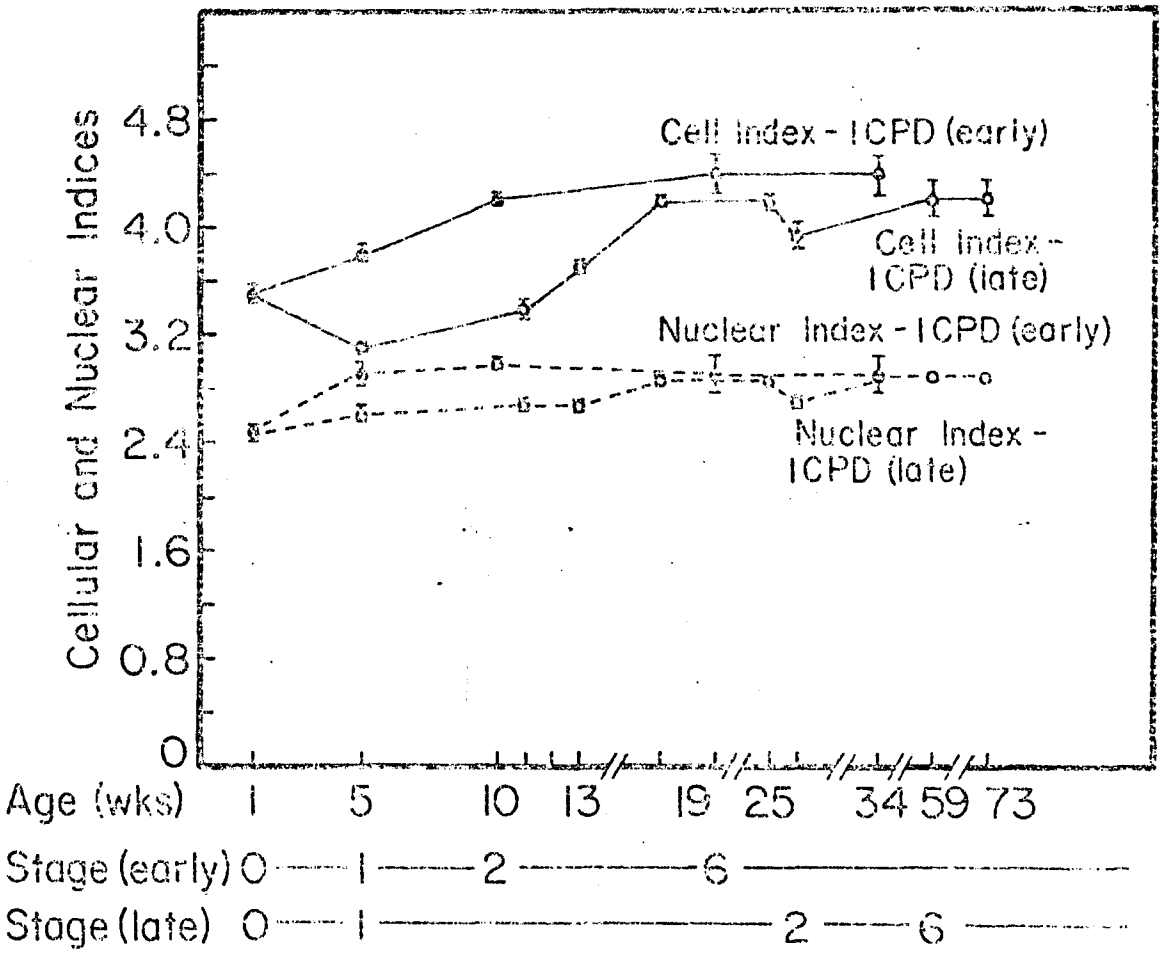


Plate 11. Cell and nuclear indices of ir-1CPD cells in early and late maturers.

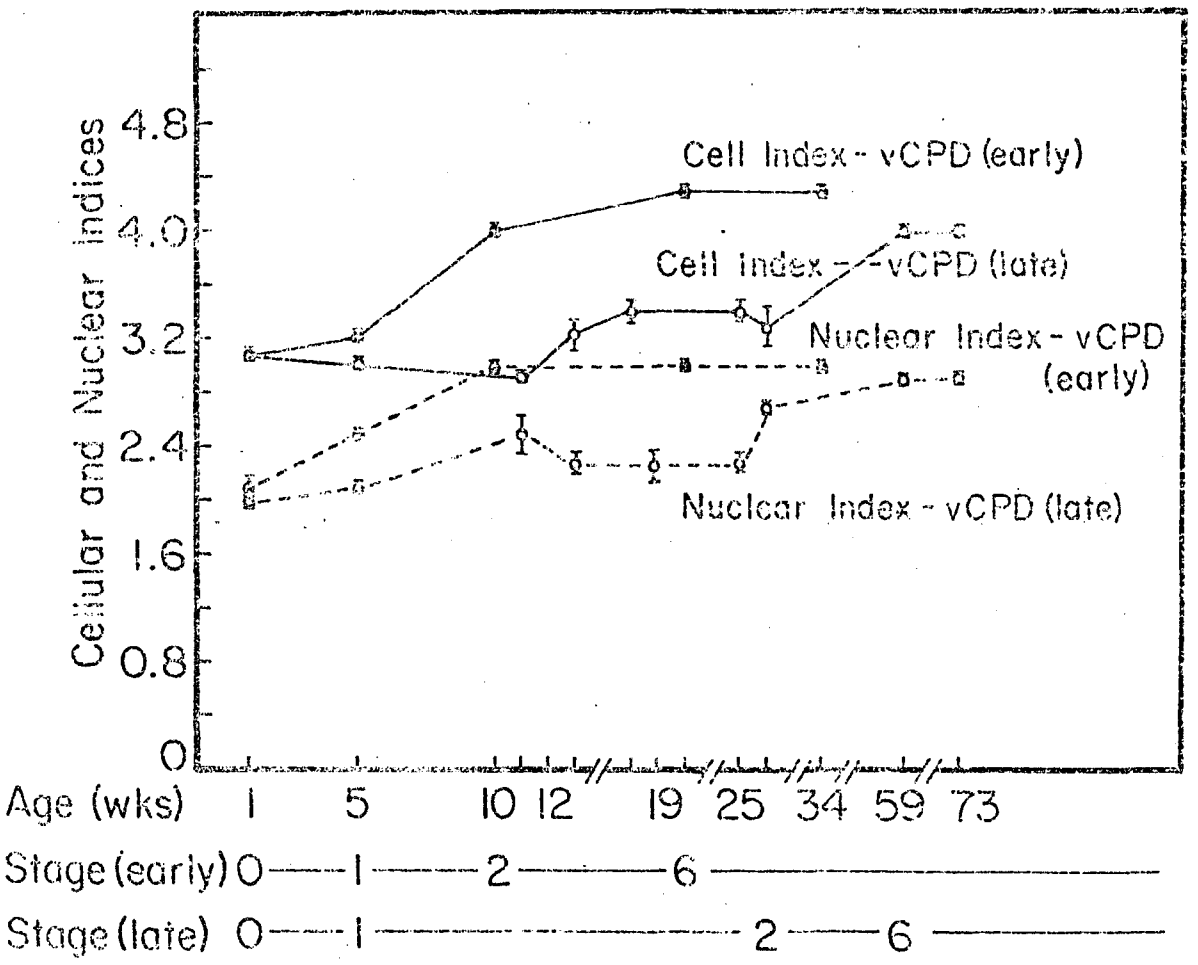


Plate 12. Cell and nuclear indices of ir-vCPD cells in early and late maturers.

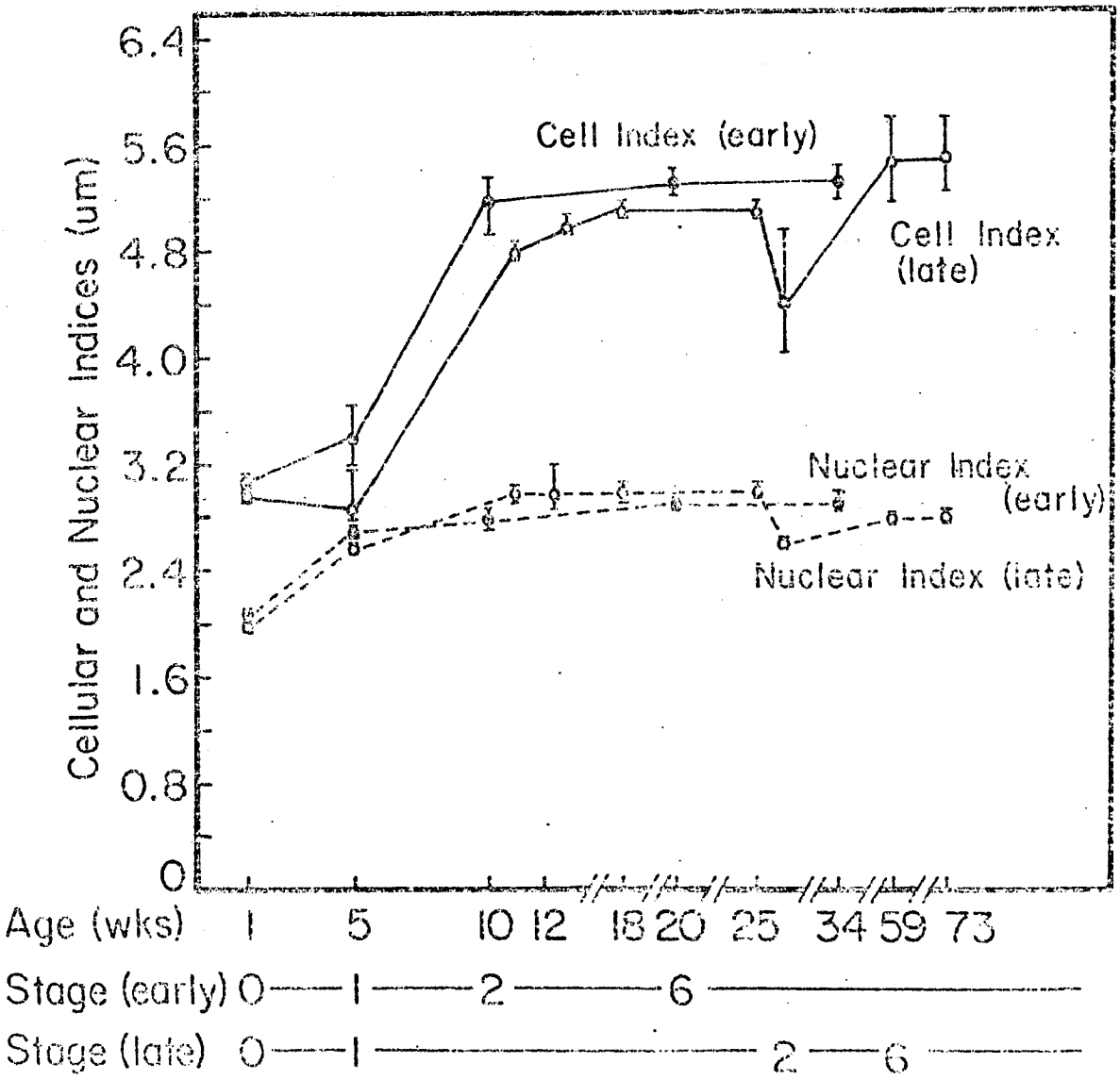
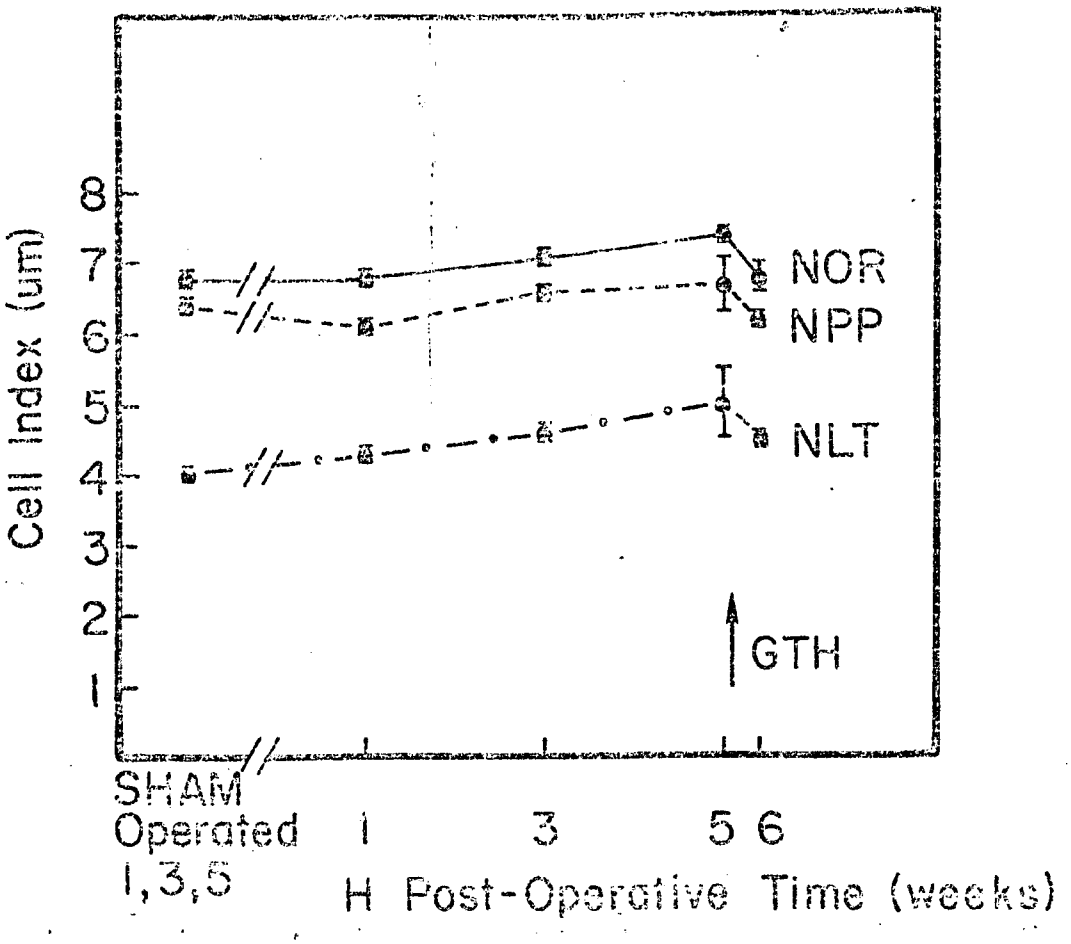
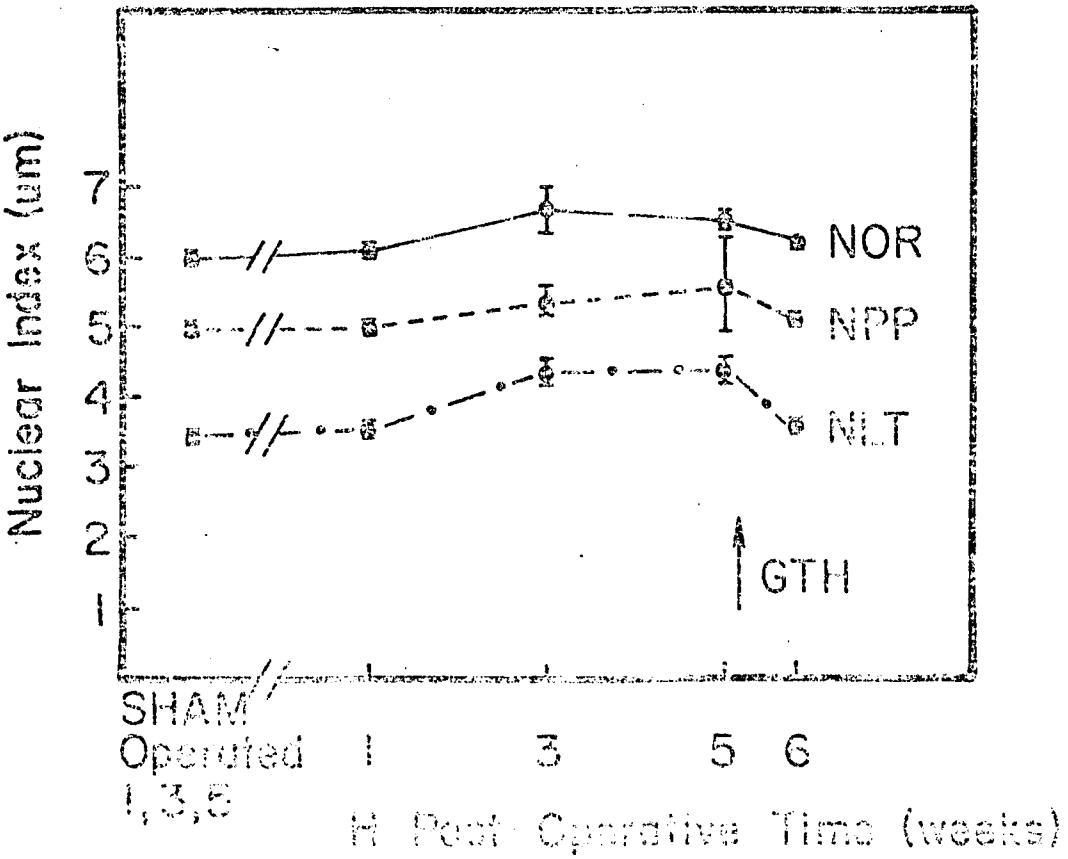


Plate 13. Cell and unclear indices of ir-PI cells in early and late maturers.

Plate 14. Cell (upper panel) and nuclear (lower panel) indices of sham-operated and hypophysectomized mature platyfish. Values for sham-operated fish are values maintained at 1,3, and 5 weeks following surgery. Post-hypophysectomy times of 1,3 and 5 weeks are indicated on abcissa. Arrow indicates time of GTH administration to those animals not sacrificed at 5 weeks post-operation time (see Materials and Methods).


Cell Index (um)
8
7
6
5
4
3
2
1
NOR
NPP
NLT
GTH
SHAM Operated 1,3,5
1
3
5 6
H Post-Operative Time (weeks)


Nuclear Index (um)
7
6
5
4
3
2
1
NOR
NPP
NLT
GTH
SHAM Operated 1,3,5
1
3
5 6
H Post-Operative Time (weeks)

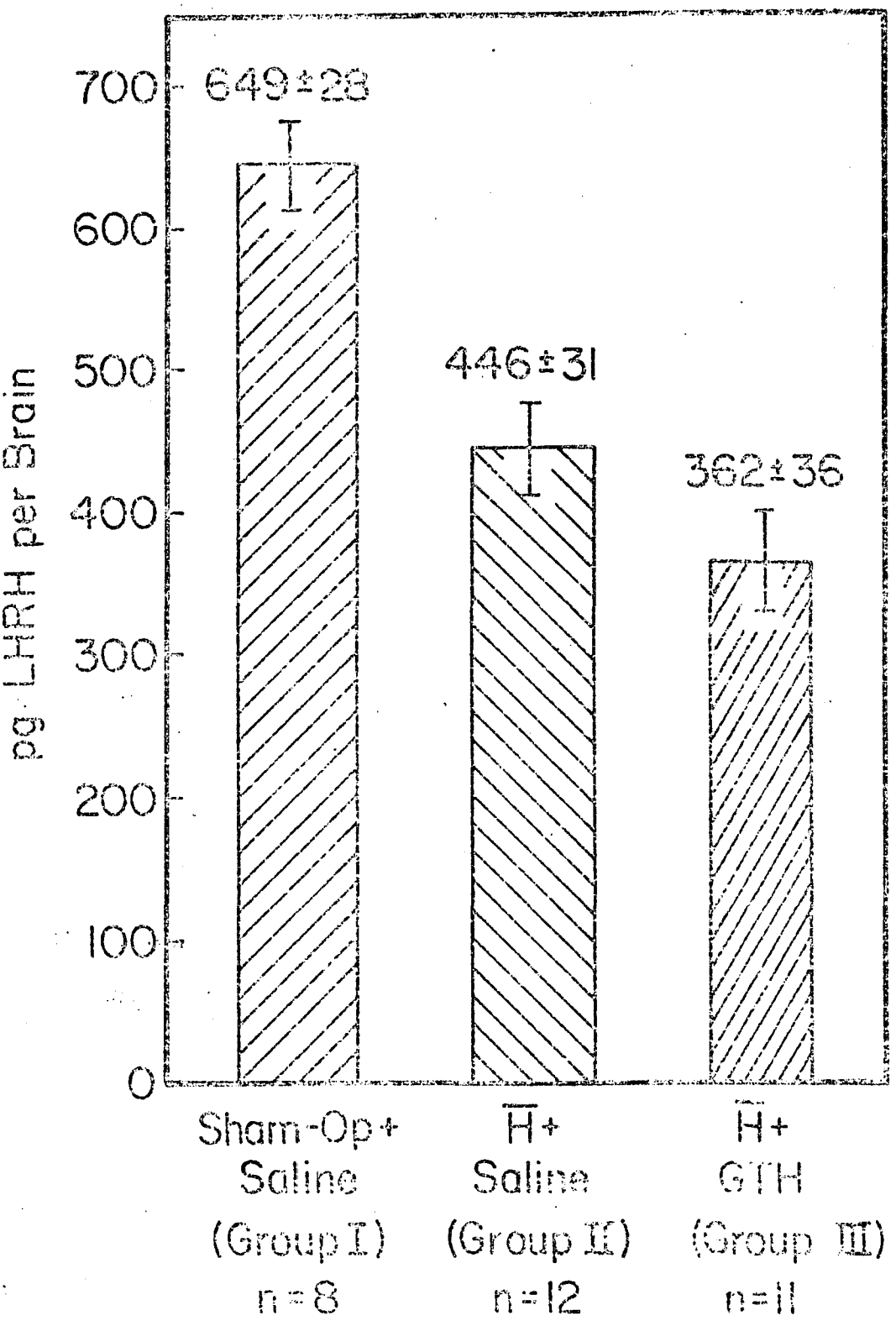


Plate 15. LHRH content per brain as determined by radioimmunoassay . Values in picograms per brain and standard error are presented for the three experimental groups.

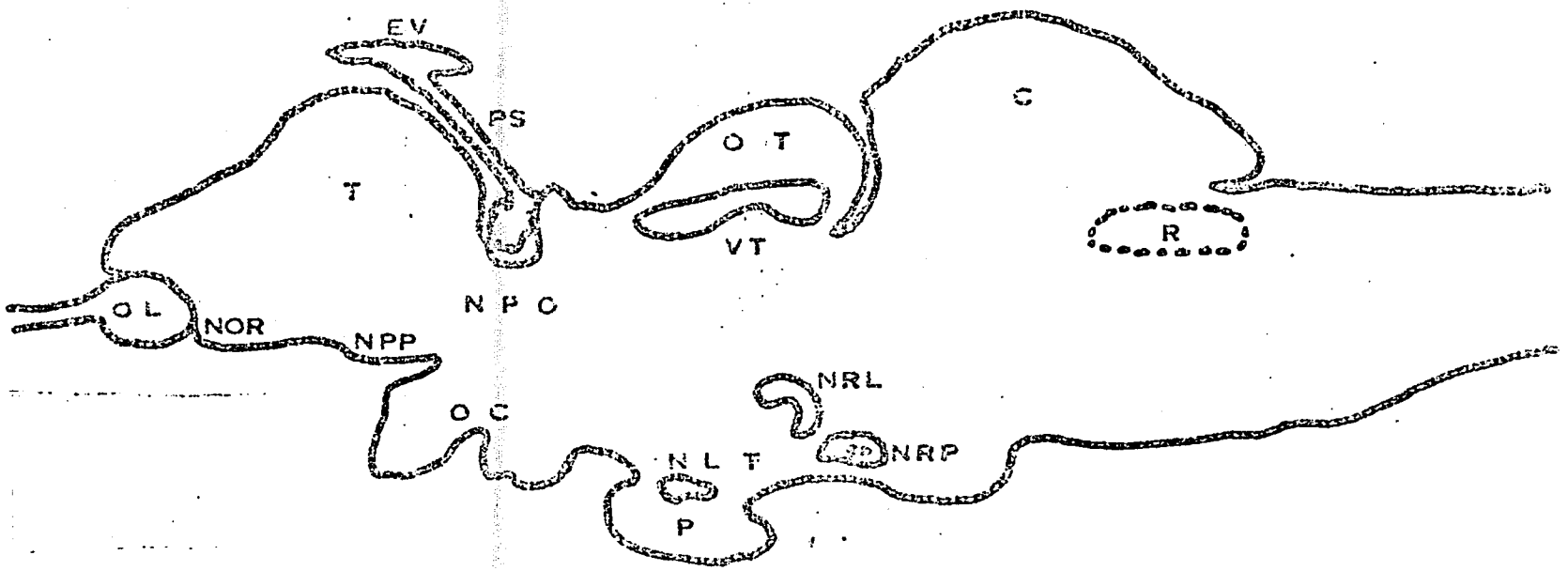
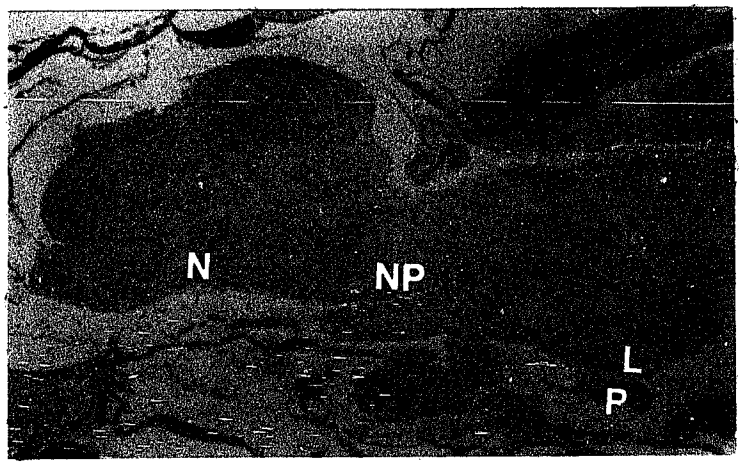


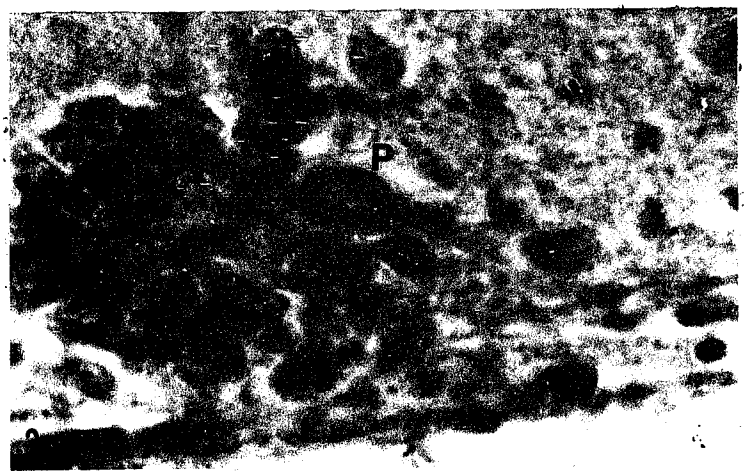
Figure 1. Line drawing of mid-sagittal section through platyfish brain and pituitary. C, cerebellum;EV, end vesicle of pineal;NLT, nucleus lateralis tuberis pars posterioris;NOR, nucleus olfacto-retinalis;NPO, nucleus preopticus; NPP, nucleus preopticus peri-ventricularis;NRL, nucleus recessus lateralis; NRP, nucleus recessus posterioris; OC, optic chiasm;OL, olfactory lobe; OT, optic tectum; P, pituitary;PS, pineal stalk; R, rhombencephalon; T, telencephalon; VT, ventral tegmentum. In all figures of the brain and pituitaries in sagittal section anterior is to the left.

Figure 2. Low power sagittal section of brain, early maturing fish, stage 6, depicts regions where ir-LHRH has been localized; reacted with anti-LHRH and counterstained with Masson's Trichrome. N(NOR), NP(NPP) , L(NLT), P(pituitary). See fig. 1. 85X.

Figure 3. NOR; early maturer, stage 6; ir-LHRH in peri-karya (P), counterstained with Kiernan. 175OX.

Figure 4. Early maturer, stage 6, reacted with anti-LHRH. Note processes with ir-LHRH radiating from NOR(N) dorsocaudally toward pineal (double arrow) and ventrorostrally (single arrow) into olfactory lobe (OL). Counterstained with Masson's Trichrome. 340X.





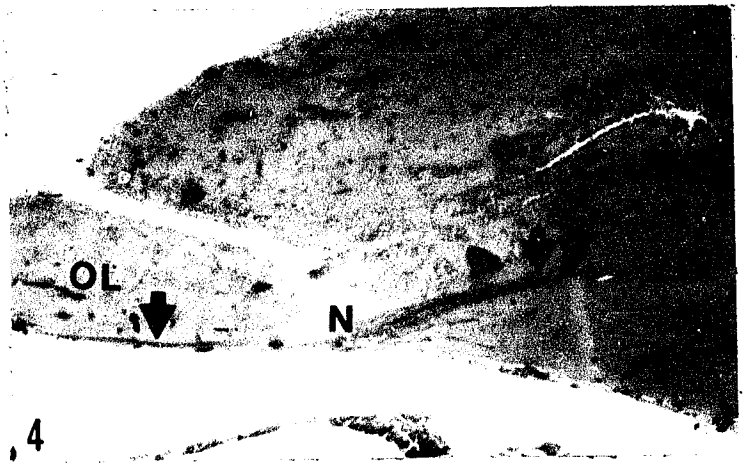


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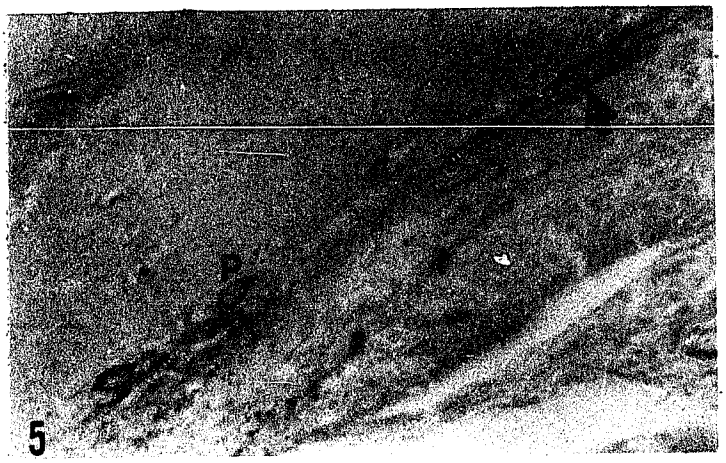
Figure 5. NPP, early maturer, stage 6. Note ir-LHRH in perikarya (P) and processes (arrow). 550X.

Figure 6. Hypophysial stalk, early maturer, stage 6. Note ir-LHRH containing processes (arrows) in hypophysial stalk. Diencephalon is upper left, pituitary lower right. 1050X.

Figure 7. NLT, early maturer, stage 6. Note ir-LHRH in perikarya (P) and fibers (arrow). 1000X.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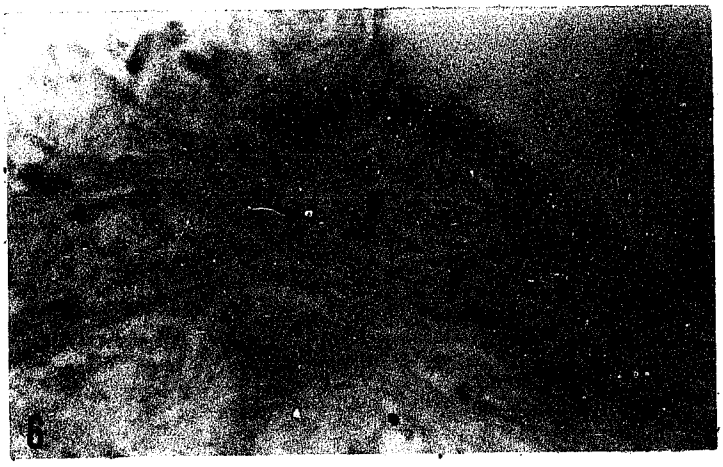
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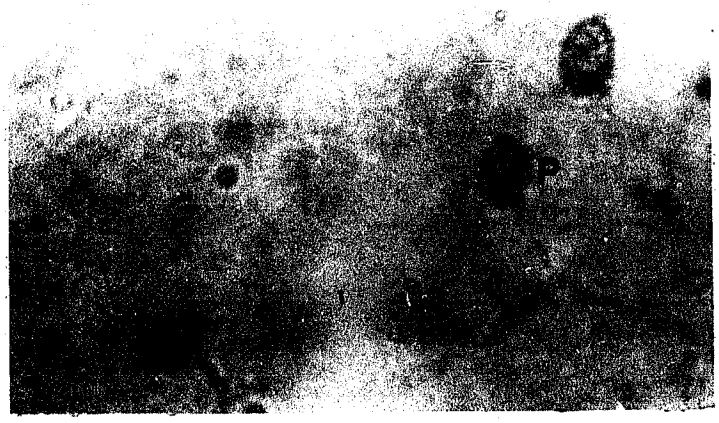
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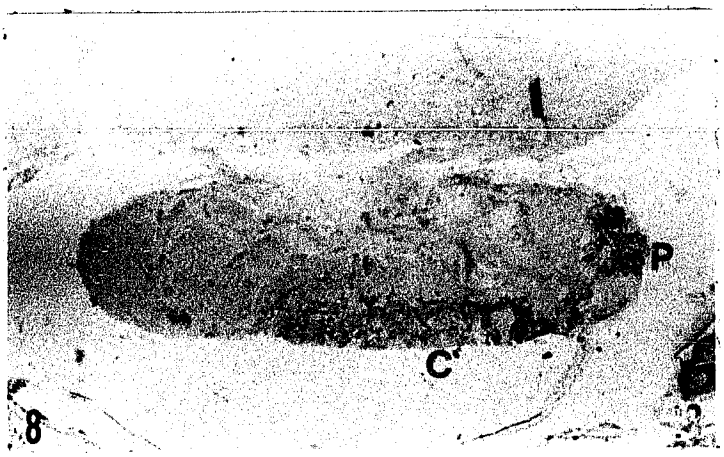
Figure 8. Pituitary, early maturer, stage 6. Note ir-LHRH in vCPD (C) and PI (P) cells. 115X.

Figure 9. NOR, early maturer, one week old. Stained with Masson's Trichrome. Note pale staining nuclei (arrows) with prominent nucleoli. 1375X.

Figure 10. Pituitary, transverse section, early maturer, one week old. Stained with anti-GTH. Note ir-cells forming lateral portions (L) and scattered on ventral border (arrow). 790X.



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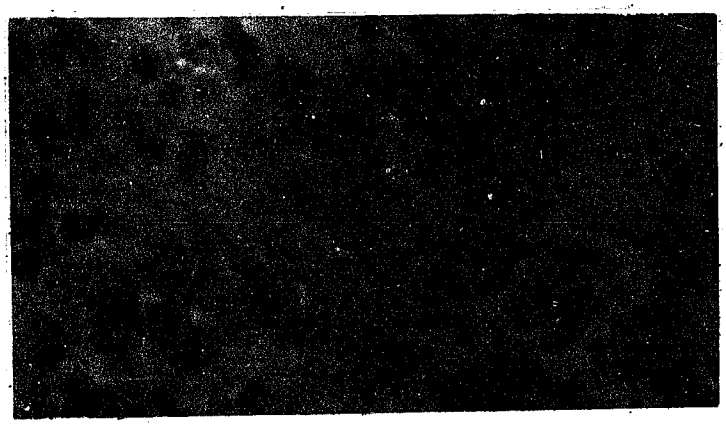

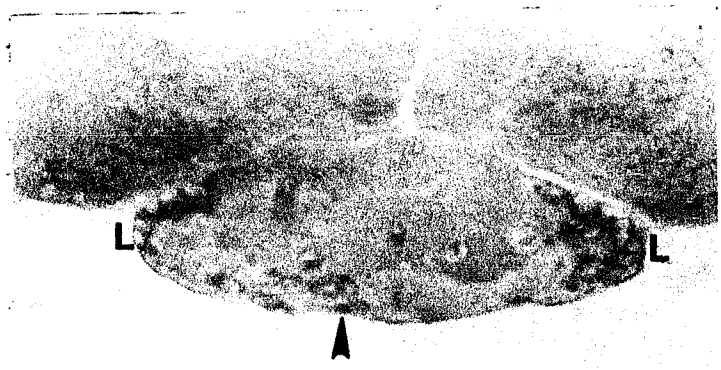

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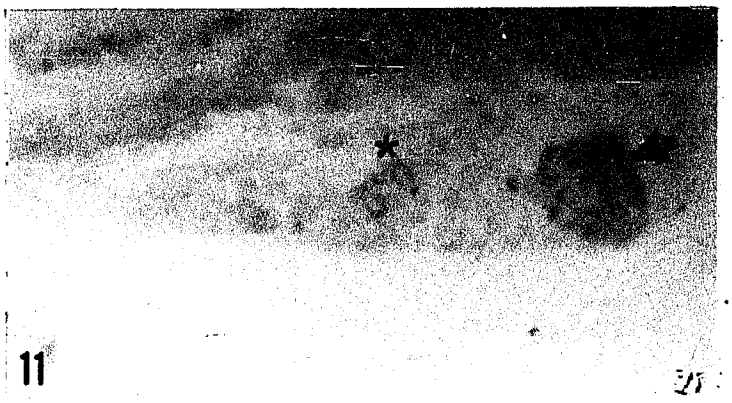
Figure 11. Pituitary, sagittal section, one week old, early maturer. Stained with anti-GTH. Note ir-material in PI (arrow) and in lateral CPD (*). 100X.

Figure 12. NOR, early maturer, stage 1, 5 weeks old. Note ir-LHRH (arrow) surrounding pale nucleus. Counterstained with Masson's Trichrome. Compare with figure 13. 970X.

Figure 13. NOR, stage 2, early maturer, 10 weeks old. Note increase in ir-LHRH perikarya when compared to stage 1 (figure 12). 1010X.

Figure 14. NPT, stage 2, early maturer, 10 weeks old. Note ir-LHRH in perikarya (P) and fibers (F). Counterstained with Masson's Trichrome. 610X.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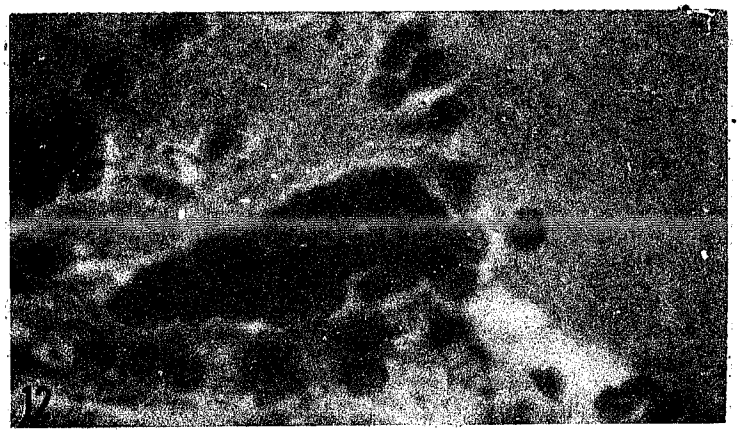
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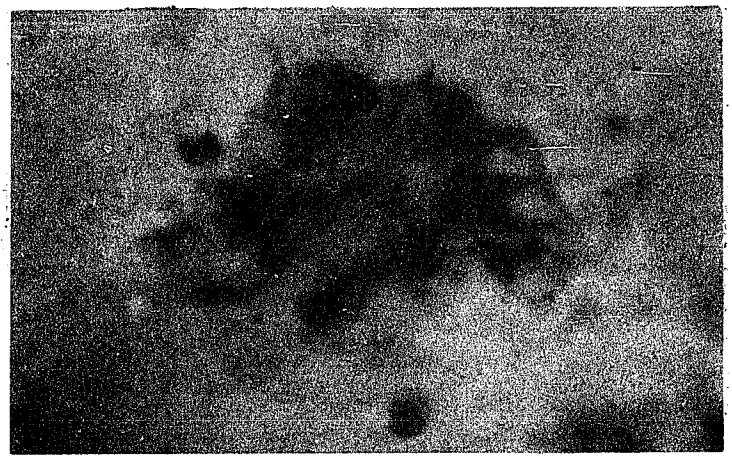

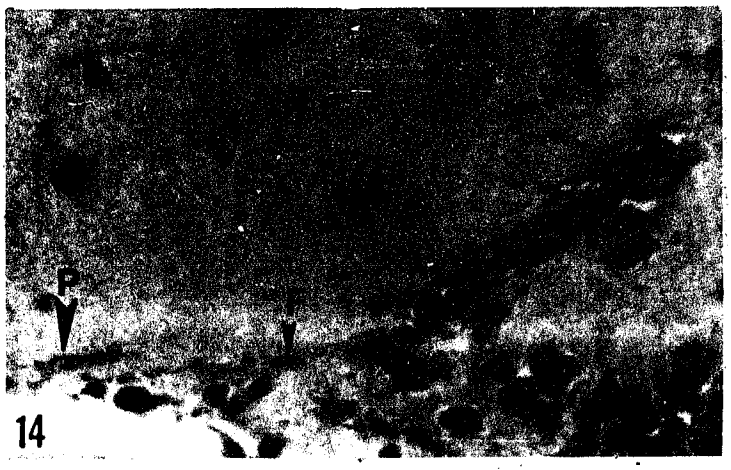
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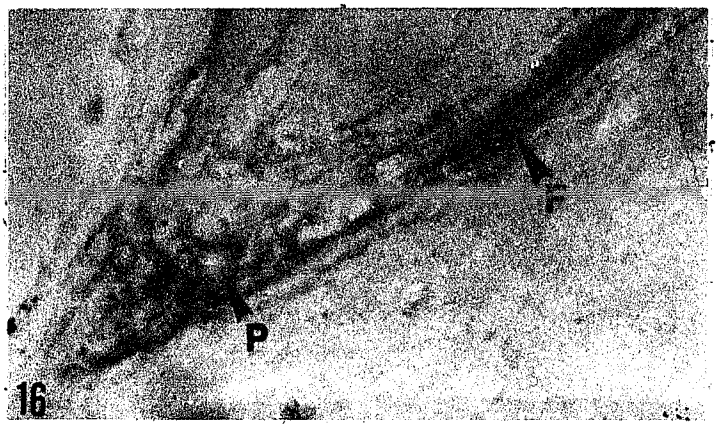
Figure 15. NMT, early maturer, stage 2, 10 weeks old. Note ir-LHRH in perikarya (P) and processes. 1690X.

Figure 16. NOR, stage 6, sham-operated fish. Note modicum of ir-LHRH in perikarya (P) and fibers (F). Compare with Figs. 17 and 18. 520X.

Figure 17. NOR, hypophysectomized saline-injected fish. Note an increase in ir-material compared to Fig. 16. 585X.

Figure 18. NOR, hypophysectomized GTH-injected fish. Note a decrease in ir-material as compared to Fig. 17. 610X.



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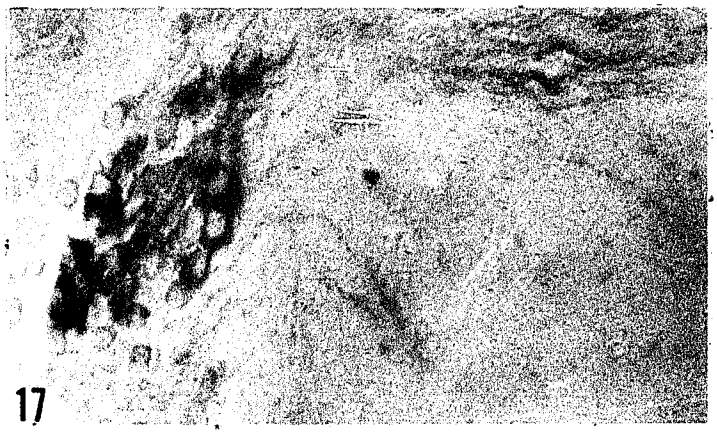
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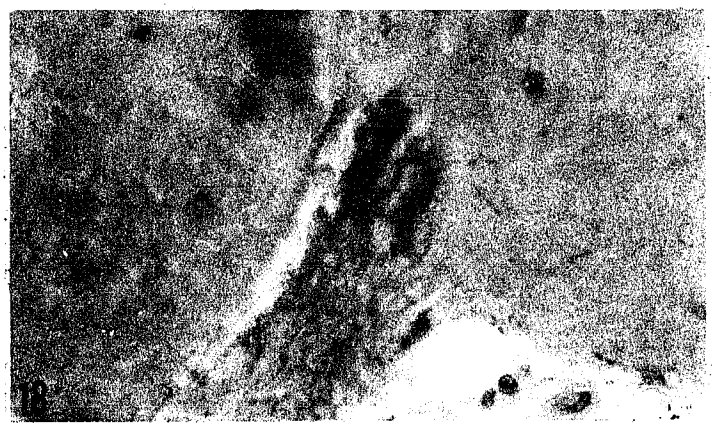
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