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A STUDY OF THE EFFECTS OF CHANGING ENVIRONMENTAL SALINITY UPON THE PROLACTIN CELLS IN A FRESHWATER TELEOST, *Xiphophorus maculatus* (THE PLATYFISH).

The City University of New York, Ph.D., 1973
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A STUDY OF THE EFFECTS OF CHANGING ENVIRONMENTAL SALINITY UPON THE PROLACTIN CELLS IN A FRESHWATER TELEOST, *Xiphophorus maculatus* (THE PLATYFISH).

by

SEYMOUR HOLTZMAN

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.

1973

This manuscript has been read and accepted for the Executive Committee in Biology in satisfaction of the dissertation requirement for the degree of Do ɔr of Philosophy.

July 26, 1973
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The City University of New York

DEDICATION

To Vicky, David, Ruth and my mother for their love, patience, support and sacrifices.

ACKNOWLEDGEMENTS

My thanks and deep appreciation to my friends at Brooklyn College and elsewhere for their aid and encouragement: first and foremost, Professor Martin P. Schreibman-no mentor could have gone further to stimulate interest and provide the facilities for successful research; my committee at Brooklyn College-Professors George H. Fried, Marion Himes and Louis G. Moriber; Professor Paul F.A. Maderson who really knows the meaning of sharing; the staff and my fellow students in the Biology Department; Professor Solomon Weinstock of the Psychology Department; Dr. Klaus Kallman of the New York Aquarium; and Dr. James Atz of the American Museum of Natural History. All of these people and many others contributed to my progress in too many ways to enumerate. I hope to repay them by doing the same for others in my future endeavors.

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LIST OF TEXT ABREVIATIONS

C.D.......Cell density = number of cells per 55 u^2

C.I.......Cell index (see page 9)

FW........Fresh water, freshwater

K.........Potassium

Na........Sodium

N.I.......Nuclear index (see page 9)

O.U.......Ocular units

(R)ER.....(Rough) Endoplasmic reticulum

RPD.......Rostral pars distalis, pro-adenohypophysis

S.........Sulfur

SW........Sea water, seawater

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INTRODUCTION

Investigation of the functional morphology of the teleost pituitary was given impetus by the observations of Pickford (1953) that the hypophysectomized euryhaline killifish, *Fundulus heteroclitus*, could not live in fresh or dilute sea water, although they survive indefinitely in full-strength sea water. Burden (1956) demonstrated that hypophysectomized *F. heteroclitus* underwent a reduction in plasma chloride concentration and osmotic pressure in dilute or hypoosmotic media. He was unable to relieve these conditions by injection of a variety of pituitary and adrenal hormones. Subsequently, Pickford and Phillips (1959) injected several mammalian hormones into hypophysectomized killifish, and found that only prolactin was effective in keeping the animals alive in fresh water. This began an interest in the biology of prolactin in fishes that is reflected by the many review articles that have appeared recently (Ball, 1969a, b; Ball and Baker, 1969; Ball, Baker, Olivereau and Peter, 1972; Bern, 1967; Ensor and Ball, 1972; Lam, 1972; Sage and Bern, 1971; Schreibman, Leatherland and McKeown, 1973).

Injections of mammalian prolactin elevate the lowered plasma electrolyte concentrations and osmotic pressure resulting from hypophysectomy in *Poecilia latipinna* (Ball and Ensor, 1967), *F. heteroclitus* (Maetz, Sawyer, Pickford and Mayer, 1967; Pickford, Pang and Sawyer, 1966; Potts and

Evans, 1966), F. kansae (Fleming and Ball, 1972), Anguilla anguilla (Maetz, Mayer and Chartier-Baraduc, 1967; Olivereau and Chartier-Baraduc, 1966; Olivereau and Olivereau, 1970), Tilapia mossambica (Dharmamba, 1970; Dharmamba, Handin, Nandi and Bern, 1967), Carassius auratus (Donaldson, Yamazaki and Clarke, 1968; Lahlou and Sawyer, 1969), Oryzias latipes (Utida, Hatai, Hirano and Kamemoto, 1971) and Ictaluras melas (Chidambaram, Meyer and Hasler, 1972). The mechanism of action of the hormone has not yet been clearly defined.

Eta cells form the bulk of the rostral- or pro-adenohypophysis in teleosts (Schreibman et al., 1973). Olivereau and Herlant (1960) were the first to suggest that they are the site of production of teleost prolactin because of the similarity of tinctorial properties of these cells in the pituitary of the European eel, A. anguilla, to the prolactin (eta)[1] cells in mammals. More definitive evidence that the eta cell is the source of fish prolactin (see discussion) comes from experiments involving incomplete hypophysectomy (Ball, 1965b, c; Schreibman and Kallman, 1966), pituitary transplants into hypophysectomized fishes (Ball, 1965a; Chambolle, 1969), the newt water-drive bioassay (Blanc-Livni and Abraham, 1970),

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[1]The term "eta cell" was originally applied to prolactin cells, prior to recognition of their hormonal product, on the basis of morphology and staining properties. "Eta cell" or "prolactin cell" will be used interchangeably for the source of the hormone, and "rostral pars distalis" (RPD) or "pro-adenohypophysis" for the area of the pituitary where these cells are found.

comparative photodensitometry of electrophoretic preparations (Clarke, 1971; Ingleton and Ball, 1972; Knight, Ingleton, Ball and Hancock, 1970), effects of pituitary extracts on blood sodium concentrations in hypophysectomized or intact fishes (Clarke, 1971; Ensor and Ball, 1968; Utida *et al.*, 1971) and immunological studies (Aler, 1970, 1971a, b; Emmart, 1969; Emmart and Mossakowski, 1967; Emmart, Pickford and Wilhelmi, 1966; Emmart and Wilhelmi, 1968; Mattheij, Stroban and Kingma, 1971; McKeown and van Overbeeke, 1969, 1971). Changes in pro-adenohypophysial cytology and ultrastructure have been correlated with changes in ambient salinity or plasma electrolyte concentrations in *A. anguilla* (Knowles and Vollrath, 1966a, b; Olivereau, 1969; Olivereau and Lemoine, 1972), *Anoptichthys jordani* (Mattheij and Sprangers, 1969), *C. auratus* (Leatherland, 1972; Leatherland and Ensor, 1973), *Cichlasoma biocellatum* (Mattheij *et al.*, 1971), *F. heteroclitus* (Ball and Pickford, 1964; Emmart *et al.*, 1966), *Gasterosteus aculeatus* (Leatherland, 1970a), *Mugil auratus*, *M. capito*, *M. cephalus* (Abraham, 1971; Blanc-Livni and Abraham, 1970; Olivereau, 1968), *O. latipes* (Nagahama and Yamamoto, 1971; Utida *et al.*, 1971), *P. formosa*, *P. latipinna* (Ball, 1969a, b; Hopkins, 1969; Olivereau and Ball, 1964), *P. reticulata* (Sage and Bromage, 1970), *T. mossambica* (Dharmamba and Nishioka, 1968), *Xiphophorus hellerii*, *X. maculatus* (Holtzman and Schreibman, 1971, 1972; Holtzman, Napoli and Schreibman, 1972), *Gillichthys mirabilis* and *Platichthys stellatus* (Nagahama, Nishioka and Bern, 1973).

The mean survival time in fresh water of hypophysectomized species of the genus *Xiphophorus* varies from 4 to 16 days, although they will live indefinitely in 1/3 sea water (Schreibman and Kallman, 1966, 1969). Hypophysectomized platyfish, *X. maculatus*, can be kept in fresh water if injected with mammalian prolactin (Schreibman and Kallman, 1966). Hypophysectomized platyfish and swordtails, *X. hellerii*, bearing ectopic pituitary grafts will also live indefinitely in fresh water, suggesting a continued secretion of prolactin by the grafts (Schreibman and Kallman, 1964). The presence of "acidophilic" cells in pituitary remnants of unsuccessfully hypophysectomized *Xiphophorus* is also associated with survival in fresh water (Schreibman and Kallman, 1966).

Prolactin cells are generally described as more "active" in fish from fresh water than in those from saline media (see discussion). Unfortunately, indiscriminate use of the word "active" provides no distinction between hormone synthesis and release. At the light microscope level, larger cells and nuclei, nucleolar prominence, and more cytoplasmic granules, chromophilia, RNA, rough endoplasmic reticulum (RER) and Golgi images are generally interpreted to be signs of synthetic activity. Recent ultrastructural studies have permitted the distinction between synthesis and release in *O. latipes* (Nagahama and Yamamoto, 1971), *G. aculeatus* (Leatherland, 1970a), *M. cephalus* (Abraham, 1971), *G. mirabilis* and *P. stellatus* (Nagahama *et al.*, 1973). Synthetic activity is usually

associated with extensive RER and Golgi elements, while evidence for secretion is suggested by exocytotic activity.

Temporal studies of prolactin cell morphology in fish under laboratory conditions have facilitated the distinction between the two types of activity. This approach has been applied to *X. hellerii* (Holtzman and Schreibman, 1972), *A. anguilla* (Olivereau and Lemoine, 1972), *O. latipes* (Nagahama and Yamamoto, 1971), *G. mirabilis* and *P. stellatus* (Nagahama *et al.*, 1973). Morphological changes of the *eta* in *X. hellerii* that are transferred from fresh water to 1/3 sea water for up to 30 days are indicative of diminished synthetic activity associated with a reduced requirement for prolactin in dilute sea water (Holtzman and Schreibman, 1972). The current series of investigations was designed to study progressive changes in the synthetic activity of prolactin cells by comparing cytologic and autoradiographic preparations of *X. maculatus* during transitional phases between fresh and dilute sea water. Several aspects of these investigations have been published in abstract form (Holtzman and Schreibman, 1971; Holtzman, Napoli and Schreibman, 1972).

MATERIALS AND METHODS

The platyfish, *X. maculatus*, is a freshwater poeciliid with a habitat that ranges from Mexico to British Honduras and Guatemala (Kallman, 1965; Kallman and Atz, 1966). Although the original stocks are from the Genetics Laboratory of the New York Aquarium, their descendants have been maintained and inbred at Brooklyn College for five to six generations. Strain 163B, originally collected in the Rio Jamapa, Mexico, in 1939, has been inbred for over 40 generations. Strain 2356 (Brooklyn College number) is descended from New York Aquarium strain 2355 produced by crossbreeding between two river populations.

A total of 259 male and female platyfish were used in these studies. The same sex and genetic strain, either siblings or fish of the same age and generation, were used in each experiment (except in experiment 4, see Table 1). The fish were kept under a 14 hour light-10 hour dark cycle, at approximately 23°C (74-76°F), in either "aged" New York City tap water (FW) or in 1/3 sea water (SW). Sea water, obtained from the wells of the New York Aquarium, was diluted to a specific gravity of 1.0075 (150mM Na, 3.3mM K). Fish were fed twice-daily on a diet consisting of liver-cereal mixture (Gordon, 1950), fresh or frozen brine shrimp nauplii, or commercial flake food (Biorell or TetraMin). All animals in the autoradiographic studies (experiments 1b-6) were fasted

for 48 hours prior to injection with tritiated-leucine. Although these fish are laboratory-bred animals, accustomed to manipulation, all were netted exactly the same number of times during the course of the experiments, to minimize the variability due to stress of handling.

The experimental animals were placed into 1/3 SW and after 21 days, 30 days or 6 months randomly selected fish were returned to FW for various durations (summarized in Table 1). FW controls were maintained and sacrificed throughout the course of the experiments.

In experiment 5a, animals received seven intraperitoneal injections on alternate days beginning with day one. The groups comprised "controls" (0.65% aqueous NaCl), "low dose" (10 µg NIH-PS8 ovine prolactin) and "high dose" (50 µg prolactin). Each dose was administered in a 10 µl volume.

FW controls and fish in 1/3 SW for 21 days in experiment 5b received intraperitoneal injections of the saline vehicle 24 hours and 6 hours before death. Animals serving as "transfer experimentals" were injected with 50 µg of prolactin twice, 24 hours prior to and again at the time of transfer to FW. "Transfer controls" were injected with the vehicle in the same schedule as the experimentals.

In experiment six, dealing with ectopic pituitary

transplants, 27 female fish were divided into three groups: a)sham-hypophysectomized and sham-transplanted; b)autotransplanted; c)sham-hypophysectomized and isotransplanted. Hypophysectomy and sham-hypophysectomy were performed by the opercular approach described by Schreibman and Kallman (1966). Transplants were made by inserting the organ into a "pocket" in the hypaxial muscle., just above the anal fin, according to the method of Kallman and Gordon (1958). Sham-transplants received a fragment of clotted blood from the site of sham-hypophysectomy. Each fish in experiment 6c received a pituitary from a FW sister. All surgery was performed one week after the fish were placed into 1/3 SW. Two weeks later, half the animals were returned to FW for six hours (except experiment 6b, see Tables 1 and 7). Completeness of hypophysectomy was determined by examining serial sections of the heads, at the termination of the experiment.

The fish were killed by decapitation and were fixed in Bouin's solution. All the heads in each experiment were processed simultaneously through decalcification ("Decal Solution", Omega Chemical Co., New York), dehydration and clearing by the Zirkle method (Krajian, 1940) and paraffin-embedding. Five micra thick sections from all the animals in an experiment were stained together in Weigert's hematoxylin and a modified Masson's trichrome procedure (Lillie, 1965). All animals and slides were coded to decrease bias in the evaluation of the results.

To determine the size of the *eta* cells, a mid-sagittal section of the proadenohypophysis was divided into quadrants. Two cells from each quadrant and two cells near the intersection of the imaginary quadrant lines were randomly selected for measurement. The two longest perpendicular diameters of the *eta* cells were measured in ocular units (1 O.U. = 11 μ) with the aid of an ocular micrometer at 2000x magnification. The mean of the two diameters of one cell was averaged with values obtained from at least nine other cells in the same gland to arrive at a "cell index" (C.I.; Leatherland, 1970a). The same method was used to calculate a "nuclear index" (N.I.). In addition, the cell density (C.D. = number of cells per 55 μ^2), was determined under an ocular grid at 1000x magnification. All cells were counted in a total of ten alternating squares, in two or three alternating bands along the rostral-caudal axis of the RPD. All measurements were subjected to one- or two-way analyses of variance and Student's or Dunnett's t tests (Steel and Torrie, 1960). Dunnett's t test was employed because Student's t test generates an excessive number of degrees of freedom when several experimental groups are simultaneously compared to a control. A significant difference between the groups was selected at the five per cent level ($p=0.05$).

Tritiated-leucine (L-leucine-4, 5-H^3; specific activity, 442.5 mCi per mg) was specially concentrated and purified for our use by the New England Nuclear Corporation (Boston, Mass.).

Purity of the labeled amino acid was radiochromatographically checked by both the manufacturer and the author. The fish were injected intraperitoneally with 20 µCi of labeled leucine in 10 µl. Thirty minutes later they were decapitated and their heads were placed into Bouin's fixative. For each animal, the two slides adjacent to the one prepared for light microscopy were stained with either periodic acid-Schiff, fast green, or a combination of both, covered with Kodak AR-10 stripping film and exposed at 4°C. The developed autoradiographs were assessed by comparing the concentration of grains (grain density) over exposures of the prolactin cells with cells on the adjacent Masson-stained slides. Labeling of the RPD was also compared with that of other pituitary areas and adjacent tissues.

RESULTS

A. Prolactin cells from fish in fresh water

The pituitary gland of the platyfish has been described in detail by Öztan (1961), Schreibman (1964) and Weiss (1965, 1967). The neurohypophysis interdigitates with three regions of the adenohypophysis which are, from anterior to posterior, the pro-adenohypophysis, meso-adenohypophysis and meta-adenohypophysis (terminology of Pickford and Atz, 1957) (Fig. 1). The pro- and meso-adenohypophysis are the equivalent of the tetrapod pars distalis, and the meta-adenohypophysis is homologous with the pars intermedia (Schreibman *et al.*, 1973). Most of the RPD, which occupies more than one-third of the adenohypophysial volume, consists of prolactin cells. The posterior border of this region is demarked by a dorso-ventral "wedge" of *epsilon* cells, which are considered to be the source of adrenocorticotrophic hormone (Fig. 2). These cells are chromophobic after Masson's staining, but have a great affinity for lead hematoxylin.

Other non-granular cells, described as "chromophobes" by Schreibman (1964) and Weiss (1965, 1967), are interspersed among the *eta* cells. A layer of chromophobes, one to three cells thick, is usually visible at the periphery of the proadenohypophysis.

Prolactin cells from FW controls are often larger than 8 µ in their longest axis. They vary in shape, but tend to appear rounded or ovoid (Fig. 3a). Nuclei are eccentric, over 4 u across, and also vary in shape. They are characterized by one or two prominent nucleoli in relatively clear nucleoplasm. A clear region, or "halo", may be detected around the nucleus. The cytoplasmic granules, which stain intensely red in the ponceau-acid fuchsin of the Masson method, are so large that the cytoplasm appears to be homogeneous rather than granular.

Heavy labeling of the RPD is clearly evident under low-power magnification after eight weeks exposure of the autoradiographic slides (Fig. 13). The density of silver grains in some areas reflects some of the larger, more intensely stained cells that are observed on adjacent Masson's stained sections (Fig. 3b).

B. Effect of 21 days in one-third sea water (Table 2)

Prolactin cells from animals in 1/3 SW for 21 days were generally degranulated (Fig. 4a), although some deeply-stained cells were present posteriorly. There were significant decreases in C.I. (15-33%) and corresponding increases in C.D. (33-57%). The largest cells did not exceed 6 to 7 u in diameter. There were statistically significant reductions (9 and 16%) in two experiments, but in the third (experiment 1b) the decrease was insignificant.

Perinuclear halos and nucleoli were not evident, and condensed chromatin could be seen. Only low radioactive labeling was found even after 12 weeks of film exposure (Fig. 4b).

Two hours after a return to FW, there were no changes detected in the *sta* cells (experiment 1c). However, by four hours (Fig. 5a) cytoplasmic staining was increased, nucleoli were again visible, and cytometric parameters were reversed from the SW condition, with 15% increases in C.I. and 13 to 17% decreases in C.D. N.I. changes were inconsistent, with a significant increase (11%) occurring only once (experiment 1a). Densely labeled cells were first observed after four hours in FW (eight weeks exposure, Fig. 5b).

Cytometric results from experiments 1a and 1b are graphically represented in figures 18 and 19.

C. Progressive changes in one-third sea water (Table 3)

Readily visible changes in prolactin cell morphology were first observed 12 hours after a transfer to dilute SW. This was manifested by degranulation and restriction of larger, intensely stained cells to the posterior and posterior-dorsal regions of the pro-adenohypophysis (Fig. 6a). There was a corresponding reduction in radioactive labeling of the RPD (Fig. 6b), and in some pituitaries the nucleoli were no longer conspicuous. Changes were not detected in the C.I.,

but the C.D. increased significantly.

Although degranulation, nucleolar regression, and reduction in radioactive labeling were more pronounced after 24 hours, they were especially marked at 48 hours (Fig. 7a), when there was a significant decrease in the C.I., as well as an increase in C.D. Radioactive labeling was low and degranulated cells were predominant. Incorporation of tritiated-leucine was apparent only after 12 weeks exposure (Fig. 7b). No cells exceeded 7.5 μ in diameter. Deeply-stained cells were still present posteriorly, but perinuclear halos and nucleoli were not visible.

After 30 days in the saline medium, prolactin cell cytology and cytometry were similar to those observed at 21 days. Significant changes in _eta_ cell N.I. were not observed at any time after transfer of the fish to 1/3 SW.

No changes were detected in any cytometric parameter by 72 hours after the fish were returned to FW from 30 days in 1/3 SW, but nucleoli, increased cytoplasmic chromophilia, and dense labeling (eight weeks exposure) were evident (Figs. 8a, b).

D. Comparisons of the effects of 21 and 30 days in one-third sea water (Table 4)

In strain 2356 females (experiment 3a), the cytometric values obtained after 21 or 30 days in 1/3 SW were similar.

prominence and increased cytoplasmic chromophilia. An increase in C.I. (8%) was first observed at 72 hours.

Autoradiography confirmed the results of experiment one in both the 2356 and 163B females of experiment three. Incorporation of tritiated-leucine was pronounced four hours after fish were returned to FW from 21 days in 1/3 SW. However, after 30 days in the saline medium, a marked increase in radioactive labeling was not apparent until 48 hours in FW, although there was an increase in grain density over the pro-adenohypophysis of some fish of both strains at 24 hours.

E. Effects of maintenance of neonatal platyfish in one-third sea water for six months (Table 5)

One week before termination of the experiment, standard length measurements were made on each fish. The mean ± SEM for FW males (23.7 mm ± 0.7) was not significantly different from SW males (23.6 mm ± 0.3). On the other hand, females in 1/3 SW (22.4 mm ± 0.5) were significantly smaller than females in FW (24.1 mm ± 0.3).

There were no differences in the cytology and cytometry of prolactin cells in both sexes, either in the FW controls (Fig. 21) or in animals returned to FW from six months in 1/3 SW. In 1/3 SW, however, *eta* cells from females were smaller and totally degranulated (Fig. 9a). In males (Fig. 10a) they exhibited some

The morphology of the *eta* cell was similar to that seen in other experiments where fish were held in 1/3 SW for 21 days (experiment 1a-c). Four hours after their return to FW, nucleoli and increased cytoplasmic staining were observed, and there was a significant C.D. reduction (experiment 3a). However, the C.I. remained unchanged in the first 24 hours after fish were returned to FW. The N.I. also increased 4 hours after a return to FW, but the value obtained at 24 hours was not significantly higher than that obtained from fish in the saline medium for 21 days.

Observations on *eta* cell morphology and cytometry from fish in 1/3 SW for 30 days confirmed those made over the same period in experiment two.

Morphologic and cytometric changes in 163B females after 21 and 30 days in 1/3 SW (experiment 3b) were similar to those described for 2356 (experiment 3a), except that no changes were detected in the N.I.

Four hours after a return to FW from 21 days in 1/3 SW, there was an increase of 15% in the C.I. and a decrease of 20% in C.D. N.I. changes were not observed. Nucleoli and increased cytoplasmic staining were evident. In contrast, after 30 days in 1/3 SW, 48 hours elapsed in FW before there was a significant N.I. increase and a C.D. decrease. The cells at 48 hours exhibited a restoration of nucleolar

pale red cytoplasmic staining, a significantly larger C.I. and N.I., and a lower C.D.

In comparison to the FW measurements, the combined results of SW males and females exhibited a decline in C.I. (33%) and N.I. (9%), and an increase in C.D. (68%). Radioactive labeling decreased in 1/3 SW (Figs. 9b, 10b), although it appeared to be slightly higher in the males.

Forty-eight hours after a return to FW, the only significant cytometric change was a 14% decrease in C.D. Nucleoli were pronounced in the small chromphobic _eta_ cells (Fig. 11), and the incorporation of tritiated-leucine was more outstanding than observed in FW controls (Figs. 12, 13). By 72 hours cytoplasmic regranulation was clearly evident, and both C.I. and N.I. increased (31% and 14% respectively). At 96 hours and seven days there were further progressive changes in the C.I. and C.D., but the N.I. were not significantly above 1/3 SW values.

F. Effects of exogenous ovine prolactin (Table 6)

There were no significant responses to exogenous prolactin injections into FW fish (experiment 5a).

In experiment 5b, both the C.I. and N.I. increased significantly in all groups returned to FW from 21 days in 1/3 SW,

while there were appropriate decreases in C.D. However, after six hours in FW, the C.D. was significantly higher for the prolactin-injected group than for the saline controls under the same conditions. No differences were detected between controls and hormone-injected fish 24 hours after a return to FW.

G. Effects of ectopic pituitary transplantation (Table 7)

There was a significant decrease in C.D. (experiment 6a), but no changes in C.I. and N.I., six hours after a return of sham-operated controls from 1/3 SW to FW. The prolactin cells in autotransplanted hypophyses (experiment 6b) behaved similarly to those from the *in situ* pituitaries of the sham-operated controls when the fish were returned from dilute SW to FW. However, the *eta* cells in the autotransplanted pituitaries, while highly variable in cytoplasmic granulation, appeared to be more synthetically active in fish in 1/3 SW for 21 days than in sham-operated animals under the same conditions. In transplants perinuclear halos could be detected and the nucleoli stood out in a relatively clear nucleoplasm.

Isotransplanted *eta* cells in sham-hypophysectomized fish (experiment 6c) were similar in morphology (Figs. 14, 16) and cytometry to the autotransplants in hypophysectomized fish. After 21 days in 1/3 SW, many of the *in situ* cells appeared to be larger and more chromophilic (Fig. 15) than those in

controls without transplants (experiment 6a), but the cytometric determinations were not significantly different. In experiment 6c the _in situ sta_ cells did not display any significant changes after the fish were returned to FW for six hours (Fig. 17).

Autoradiographic evidence is not available for experiment six.

DISCUSSION

A. Methods

Experimental Animals

The platyfish, *X. maculatus*, offers several advantages as a laboratory animal. Fishes of the genus *Xiphophorus* have long been successfully maintained in home aquaria because of the relative simplicity of their requirements. Their small size, rapid growth, early maturation and fecundity insures a large supply of experimental animals throughout the year. The The platyfish pituitary is ideal for studying prolactin cytophysiology because the *eta* cells, which are restricted to the RPD, are not mixed with other hormone producing cells.

The use of inbred laboratory strains for physiological studies may reduce genetic variability (Falconer, 1967). Utilization of two strains of platyfish was necessitated in these studies by an unusual reduction in breeding activity during the course of the experiments. Thus an opportunity was provided to compare the experimental responses of different genotypes. In spite of their genetic differences, the two strains of platyfish yielded similar results. Since the specific origin of the 163B fish is known it is also possible to compare this highly inbred strain to the natural population.

Cytology and Cytometry

Although a number of fixatives may be used to prepare pituitaries for cytological studies (Ball and Baker, 1969), Bouin's fixation was selected for the following properties: it is an excellent protein fixative for the autoradiographic demonstration of tritiated amino acids (Gude, 1968); it penetrates rapidly; it partially decalcifies; and it eliminates several steps required with other methods, such as washing, or treatment with iodine and thiosulfate (which may also interfere with autoradiography). The exclusive use of Bouin's obviated a discussion of fixation artifacts introduced by a variety of fixatives. Schreibman (1964) objected to fixatives containing acetic acid because of their effects on meso-adenohypophysial cyanophils, but in our hands, Bouin's fluid, in conjunction with a modified Masson's stain, allowed the tinctorial distinction of all known cell types.

Cytometric methods were used to detect those changes which may be more subtle than the abrupt alterations that can be seen by comparisons of cell morphology. Analysis of activity based only on the tinctorial properties of hypophysial cells may be misleading. Their supplementation by cytometry has been recently discussed by Leatherland and Ensor (1973).

The use of two approaches, the C.I. and C.D., for determining

changes in cell dimensions is an accepted procedure for the analysis of pituitary cytology (Leatherland, 1970a). One would expect these parameters to be inversely related, and in most cases they are. However, C.D. determinations are probably more reliable than C.I. because more cells (2-3x) are involved in each determination. In order to reduce regional bias, objective methods of selecting cells were devised (see materials and methods), but the C.I. may also suffer from a tendency to select larger, more intensely stained cells for measurement.

Nuclear measurements have been used as indicators of eta cell activity in C. auratus (Leatherland, 1972), C. biocellatum (Mattheij et al., 1971), G. aculeatus (Leatherland, 1970b), M. auratus (Olivereau, 1968), P. formosa (Olivereau and Ball, 1966) and P. latipinna (Ball, 1969a; Ball et al., 1972). An analysis of the data suggests a reduction of nuclear size in X. maculatus in 1/3 SW, and an increase upon a return to FW, but the N.I. has not been found to be a reliable indicator of cellular activity because of the inconsistencies among experimental groups. The shortcomings of this method may reside in purely technical difficulties. A thin section through the center of a relatively large prolactin cell may leave only a portion of the eccentrically placed nucleus, yielding erroneous values. Perinuclear halos and densely-stained cytoplasmic granules that overlie the nuclei may obscure the nuclear boundaries. Even without these limitations, it may be

impossible to detect small changes in the inherently small nuclei. In this instance, examination of nuclear morphology is probably a more effective means of evaluating activity than a cytometric approach.

Autoradiography

Autoradiographic studies of the teleost pituitary were pioneered by Deminatti (1962a, b, 1963, 1964) and Leray (1963). The pro-adenohypophysis showed marked incorporation of ^{35}S-cysteine in M. cephalus (Leray, 1963), and both ^{35}S-methionine and ^{3}H-phenylalanine in C. auratus (Deminatti, 1962a, b). Deminatti (1964) demonstrated that the RPD of the FW goldfish is densely labeled by ^{3}H-acetate, suggesting high synthetic activity in cells previously shown to incorporate labeled amino acids. Ball (1969a) interpreted the incorporation of labeled acetate to be a sign of protein synthesis. This interpretation may be oversimplified because acetate is also a metabolite in lipid and carbodydrate metabolic pathways (Deminatti, 1964). The use of tritiated-leucine offers several advantages: the low energy β emissions of tritiated compounds make them the precursors of choice for autoradiographic localization studies (Gude, 1968); leucine is not known to be incorporated into anything but protein; and approximately 12.5% of ovine prolactin residues are leucine (Li, 1969). Ovine prolactin has three disulfide bonds that

are essential for its biological activity (Li, 1969). In this regard, the use of ^{35}S-labeled amino acids may provide an additional tool in autoradiographic studies. Eta cells from Xiphophorus sp. (Sage, 1968), A. anguilla (Olivereau, 1963) and M. cephalus (Leray, 1966) are histochemically positive for sulfhydryl or disulfide groups, but a failure to stain the cells in these and other species by alcian blue has led Ball and Baker (1969) to suggest that the cysteine content is low.

The injection of labeled amino acids into intact animals for subsequent autoradiography places some limitations upon the interpretation of results. One must be able to distinguish changes in "uptake" from "incorporation". Chase experiments might be used to determine amino acid pool size or the rate of release of a secretory product. When incorporation is low, longer exposures are needed to provide enough particle hits to cause observable grain deposition (Gude, 1968). After eight weeks of exposure, the film over the eta cells from fish in 1/3 SW frequently appears to be devoid of grains, but exposure for 12 weeks demonstrates that leucine incorporation still occurs. Autoradiographic studies should be supplemented by biochemical and physiological investigations, especially since changes of incorporation rate per se provide no information on the nature of the product. Such methods also provide more accurate quantitative information on synthetic activity. However, a comparison of the autoradiographs with the

cytology of adjacent sections, stained for light microscopy, offers some basis for evaluating changes in the synthesis and possibly differential release of hormonal products. The value of correlating autoradiography with cytology is especially evident where the autoradiographic picture may be the same, but cytology indicates that the cell is doing different things. In FW controls, grain density (the number of grains per unit area) after eight weeks of film exposure is high over the RPD, and the cytology suggests an accumulation of secretory product. Labeling is similar in fish returned to FW from 30 days or longer in 1/3 SW, but the cytology after 24 to 48 hours suggests that the secretory product is not being accumulated (experiments 2-4).

B. Comparative aspects of prolactin cell histology in fishes

The platyfish is typical of most teleosts in that the prolactin cells are irregularly arranged and form the bulk of the RPD. In the isospondylous Anguillidae, Clueidae and Salmonidae these cells are grouped in follicle (Olsson, 1968). Heteropneustes fossilis may be exceptional in that its prolactin cells are scattered throughout the meso-adenohypophysis (Sundararaj and Nayyar, 1969). Prolactin has also been identified by immunohistochemical methods in cells scattered throughout the pars distalis of two freshwater Brachyopterygian fishes, Polypterus palmas and Calamoicthys calabaricus (Aler, 1971b).

Eta cells may vary in shape from columnar in species with follicular pro-adenohypophyses to polyhedral or round in other fishes. The distribution of organelles and inclusions also varies among the prolactin cells of different teleosts. Regardless of these interspecific variations, the cells share affinities for erythrosin, azocarmine and acid fuchsin, and are negative to PAS, alcian blue and aldehyde fuchsin. Prolactin cells are usually distinguished from the meso-adenohypophysial somatotrophs by their location. The somatotrophs also have an affinity for orange G when stained by the Azan method, or are colored yellow when processed through the alizarin blue tetrachrome method, whereas the prolactin cells are stained red by these procedures (Ball

and Baker, 1969; Van Oordt, 1968).

C. *Eta* cells in platyfish in fresh water

A combination of large cell size, prominent nucleoli, perinuclear halos and an abundance of intensely-stained secretory granules reflect synthetic activity in the prolactin cells of *X. maculatus* in fresh water. This is confirmed by the large number of grains over these cells after only eight weeks of autoradiographic film exposure. A similar description of *eta* cell morphology has been reported for *X. hellerii* (Holtzman and Schreibman, 1972). In *X. hellerii* and *X. maculatus* the perinuclear halo is composed of several concentric layers of rough endoplasmic reticulum (RER) that contain large cisternae (Holtzman and Schreibman, 1972; Weiss, 1965, 1967), and a similar arrangement is found in *M. cephalus* (Abraham, 1971), *O. latipes* (Nagahama and Yamamoto, 1972) and *T. mossambica* (Dharmamba and Nishioka, 1968).

Large prolactin cells are frequently found in proximity to blood vessels in *Xiphophorus* (Holtzman and Schreibman, 1972). These vessels have been described as sinusoids, rather than capillaries, because of their relatively large size (Schreibman, 1964). Direct contact with a sinusoid is probably not a prerequisite for the discharge of secretory material from the prolactin cell. Weiss (1965) has presented ultrastructural evidence that granules are released either into the intercellular

space or into the chromophobes before entering the blood. In *G. aculeatus*, capillaries are peripheral in distribution and are surrounded by degranulated cells which are interpreted to be in a state of higher "secretory activity" (Schreibman *et al.*, 1973). Areas close to capillaries in *Oncorhynchus nerka* are also less granulated (McKeown and van Overbeeke, 1969). The autoradiographic methods used in the current studies might reveal whether these represent areas of differential synthesis and release in *Gasterosteus* and *Oncorhynchus*.

The nature of the non-granulated cells that are distributed among the prolactin cells and at the periphery of the RPD is a source of speculation. These cells have been called "chromophobes" (Schreibman, 1964; Weiss, 1965, 1967), "neck cells" (Knowles and Vollrath, 1966b) and "channel cells" (Abraham, 1971). In addition to their possible role as transport systems for the secretory product (of the prolactin cells), as discussed above, it has been speculated that they may be a source of new prolactin cells (Schreibman *et al.*, 1973).

D. *Eta* cells in platyfish in dilute sea water

Early degranulation and loss of visibility of the perinuclear halos in the prolactin cells of *X. maculatus* in 1/3 SW agrees with previous light and electron microscopic observations on *X. hellerii* (Holtzman and Schreibman, 1972).

In contrast, the *sta* cells of the eel, *A. anguilla*, do not show degranulation for at least five days following its gradual introduction into full-strength sea water (Olivereau and Lemoine, 1972). In the eel, morphological responses of the prolactin cells are much slower and, in general, not striking for the first 30 days in SW. In spite of the difference, the earliest degranulation appears to be peripheral in both *Xiphophorus* and *Anguilla*. In the only other report of early changes in a saline environment, Olivereau and Ball (1970) observed that prolactin cells and nuclei become significantly smaller, with "less pronounced nucleoli and Golgi images", by 72 hours after *P. latipinna* are transferred from FW to 1/3 SW.

Table eight summarizes the changes in the prolactin cells of teleosts, that are associated with elevated ambient salinity. A reduction in cell size may reflect a reduction in the volume of the cytoplasm, nucleus, mitochondria, endoplasmic reticulum and the Golgi elements. This would also result in diminished protein synthesis and accumulation of secretory material. Supporting evidence comes from the low incorporation of tritiated-leucine observed in the autoradiographs of *sta* cells from platyfish in 1/3 SW. This evidence contrasts with the increases in the rate of tritiated-leucine incorporation detected by biochemical studies on the gill, kidney, liver and skeletal muscle from *X. maculatus* in dilute SW (Holtzman and Schreibman, 1970 and unpublished data).

A sound evaluation dictates the use of several criteria as indices of cellular activity. The use of any single parameter is hazardous because of intra- and interspecific variations that may occur. For example, _eta_ cell size does not change in _A. jordani_ after four weeks in 20% SW, although the cells degranulate and the nucleoli are "small and inconspicuous" (Mattheij and Sprangers, 1969). Similar problems exist with the interpretation of cytoplasmic chromophilia in the absence of other parameters. In _C. biocellatum_ there is no evidence of degranulation after four weeks in 25% artificial SW, although the pro-adenohypophysis, _eta_ cells, nuclei and nucleoli are smaller (Mattheij _et al._, 1971). The interpretation of the observations on _A. jordani_ and _C. biocellatum_ in dilute SW is that the prolactin cells exhibit "reduced activity" (Mattheij _et al._, 1971) but the authors do not elaborate on the nature of the "activity".

The tinctorial characteristics of the prolactin cell depend upon the number, size and chemistry of the cytoplasmic granules. In teleosts, secretory granule size is generally reduced when the environmental salinity is increased (Schreibman _et al._, 1973). After 30 days in 1/3 SW, the _eta_ cells of _X. hellerii_ still contain small granules, although the cells are chromophobic (Holtzman and Schreibman, 1972). In _A. anguilla_, the cytoplasm contains cyanophilic granules after five months in SW, and there is a marked affinity for lead hematoxylin that is not present in FW animals

(Olivereau, 1970; Olivereau and Lemoine, 1972). Olivereau (1970) suggested that this supports the concept of Knowles and Vollrath (1966b) that the small granules which are present in marine *Anguilla* represent another principle which is necessary for the eel in a saline environment. Other investigators have suggested that small granules are the result of "residual" or "continued but impaired" synthesis of prolactin (Abraham, 1971; Dharmamba and Nishioka, 1968; Holtzman and Schreibman, 1972; Nagahama and Yamamoto, 1971). This interpretation is supported by the unimodal distribution of granule sizes in the prolactin cells of FW and SW fishes (Dharmamba and Nishioka, 1968; Holtzman and Schreibman, 1972).

Nicoll (1972) proposed that prolactin may exist in the *eta* cell in two forms, large "presecretory" granules and smaller "storage" granules. Tinctorial or size characteristics could, therefore, depend on differences in the physico-chemical state of prolactin in the cells of FW and SW fishes. These differences may also be exhibited by the affinity of electrophoretically-treated pituitary extracts for tetrachrome stains, although the use of these stains for electrophoretic preparations has not been reported.

The presence of prolactin in the *eta* cells of fishes in salt water has been demonstrated by immunological methods in *F. heteroclitus* (Emmart *et al.*, 1966), *O. nerka* (McKeown and van Overbeeke, 1969, 1972) and *Pollachius virens* (Emmart and

Bates, 1968; Emmart and Wilhelmi, 1968). Bioassays that demonstrate sodium-retaining activity in pituitary extracts from fish in salt water are presumed to reflect the presence of prolactin. This activity is lower in extracts from SW fishes than from those in FW (Clarke, 1971; Ensor and Ball, 1968; Utida et al., 1971). Evidence for the continued synthesis of prolactin in SW should be obtained by extending the radioactive labeling methods developed in the current investigations to ultrastructural and biochemical studies. Until such investigations are undertaken, it can only be assumed that reduced incorporation of radioactive leucine represents a continued low level of hormone synthesis. It may be that most of the amino acid is actually going into the production of other molecules, such as enzymes. For example, Hopkins (1969) has reported an increase in acid esterase content in the prolactin cells of P. latipinna in dilute SW.

Autoradiographic and cytologic studies of prolactin cells suggest that release continues independently of synthesis in the platyfish in dilute SW. By 12 hours the number of grains over the RPD is uniformly low, but degranulation and chromophobia continue progressively thereafter. Here again species differences may be noted. An increase in the salinity of the eel's environment may temporarily inhibit prolactin release, while synthesis and accumulation of the hormone continues (Aler, 1971a; Olivereau

and Lemoine, 1972). Abraham (1971) suggested that prolactin release is increased, but synthesis is reduced, in M. cephalus with rising ambient salinity. However, in hypersaline lagoons "the ER suggests heightened activity, while the extremely reduced volume of the RPD, the decrease of cellular size in comparison with FW specimens...and the scarcity of secretory granules could be the result of high release rate without a sufficient increase in synthetic activity". These proposals were based upon electron microscopic observations on Mugil that were taken from different biotopes. However, evidence from time studies does not support a concept of increased rate of release in SW. For example, in X. hellerii transferred from FW to 1/3 SW there is a marked decrease in granule size within 18 hours, but no sign of release (Holtzman and Schreibman, 1972). In several species, cytoplasmic granule accumulation in salt water is followed by both rapid degranulation and increased exocytotic activity upon entry into FW (see next section of discussion). It is difficult to understand the physiological value of increasing the rate of prolactin release in elevated salinities when the major role of this hormone appears to be the adaptation to a hypoosmotic environment. An alternative to Abraham's explanation of the increased endoplasmic reticulum in Mugil from hypersaline lagoons is the possibility that unusually high salinity may stimulate the synthesis of proteins other than prolactin, as previously discussed.

A reduction in nuclear size, associated with elevated ambient salinity in most species (Table 8), was not detected in X. maculatus, O. latipes (Nagahama and Yamamoto, 1971) and T. mossambica (Dharmamba and Nishioka, 1968). Condensed chromatin and attenuated nucleoli are the outstanding nuclear characteristics of the eta cells in platyfish in 1/3 SW. This nucleolar reaction has been reported for most species. The condensed chromatin, in a relatively clear nucleoplasm, cannot be attributed to pycnosis. These changes suggest that transcriptional activity is depressed in the eta cell, and this concept is supported by reports of reduced histochemically-detectable cytoplasmic RNA in salt water fishes (Ball, 1969a; Olivereau and Ball, 1970). Since this may only represent dispersal and dilution of the RNA within the cytoplasm, radioactive nucleic acid precursors should be used to quantitatively and qualitatively verify turnover rates of these molecules in different salinities.

There is no evidence of sexual dimorphism in the eta cells of X. maculatus in FW (Schreibman, 1964), nor has any been presented for other species. Experiment seven suggests that differences in the prolactin cells may be uncovered only after long-term exposure (six months) of the platyfish to elevated ambient salinity (experiment 7). The eta cells of the males in 1/3 SW were larger and more chromophilic than those of the females. In the males they may either be inhibited from releasing the small amount of hormone that is

being synthesized, or the rate of synthesis may exceed the rate of release. The results of this investigation should be confirmed because of the limited number of animals that was used.

The multiplicity of functions for prolactin in teleosts has been reviewed by Bern and Nicoll (1968) and it is clear that the predominant role is osmoregulation. Other functions may be revealed in dilute SW in the presence of low circulating levels of prolactin. One of these may be growth. Although the standard length of freshwater- and dilute seawater-reared males is similar, freshwater females are significantly longer than those in sea water (experiment 7). Cell and nuclear indices of the somatotrophs from females in FW (C.I.=0.47 $\pm$ .30; N.I.=0.27 $\pm$ .01) and females in 1/3 SW for six months (C.I.=0.45 $\pm$ .26; N.I.=0.30 $\pm$ .01) are not significantly different, suggesting that this is not due to the action of growth hormone. It is well established that prolactin and growth hormone have similar molecular structures (Bewley, Dixon and Li, 1972), and prolactin has been reported to have growth-promoting effects in tadpoles (Bern, Nicoll and Strohman, 1967), juvenile lizards (Licht and Hoyer, 1968) and mammals (Weldon, Jacobs, Pagliara and Daughaday, 1972). Although the results suggest that teleost prolactin may have growth-promoting effects, there is a dearth of information on this point. Mammalian prolactin does not induce growth of hypophysectomized F. heteroclitus (Pickford and Kosto, 1957), although it "alleviated the shrinkage" of hypophysectomized

P. *latipinna* (Ball, 1969b).

Other environmental factors may influence prolactin cell responses to elevated salinity. In marine G. *aculeatus* the primary factor may be the photoperiod rather than salinity since synthesis and secretory granule accumulation occur in the early spring, prior to anadromous migration. However, in the laboratory, prolactin cell size is reduced when late spring *Gasterosteus* is transferred from FW to SW (Leatherland, 1970a), but changes in other cell characteristics have not been reported. Sokol (1961) has described seasonal changes, associated with the reproductive cycle, in the size and chromophilia of the *eta* cells in marine F. *heteroclitus*.

E. Prolactin cell activity in fresh water following different periods in dilute sea water

Cytology, cytometry and autoradiography of the prolactin cells in X. *maculatus* suggest the recovery of synthetic activity within four hours after a return to FW from 21 days in 1/3 SW (experiments 1 & 3). There are no signs of activation by two hours, but such evidence should be sought at the ultrastructural level. Nagahama and his coworkers (1971, 1973) did not observe modifications of the *eta* cells of O. *latipes* and G. *mirabilis* at the light microscopic level within the first three hours after a transfer to FW, although ultrastructural changes, including exocytosis,

development of RER, mitochondria and Golgi elements, and the formation of new granules in the Golgi system were detected after the first half hour.

Platyfish were kept in 1/3 SW for 30 days before a return to FW (experiment 2) because of the presence of deeply-stained cells at 21 days. Although there are no apparent morphological differences between the *eta* cells from the two strains of fish in dilute SW for 21 or 30 days (experiments 1-4), there evidently is a profound physiological difference. A return to FW from 30 days, or even six months, in 1/3 SW requires 24 to 48 hours before signs of recovery are observed. This suggests that the saline environment exerts a maximal effect upon the synthetic mechanisms of the prolactin cell during a critical period between 21 and 30 days. As far as can be determined, this phenomenon has not been previously reported. The reason for the relatively long period before the effect is detected is puzzling, and probably depends upon subtle biochemical or physico-chemical changes that are not reflected in cellular morphology.

Nagahama *et al.*(1973) suggest that the adjustment of prolactin cells to FW is much slower in euryhaline marine teleosts than in euryhaline FW fishes. Although prolactin synthesis appears to be activated within hours after a transfer from SW to FW, an accumulation of secretory granules is not observed in *G. mirabilis* by 30 days, or in *P. stellatus* by 10 days. In *Poecilia* the *eta* cells resemble those in FW

fish by seven days after a transfer from 1/3 SW to FW (Ball, 1969a). The data from _X. maculatus_ are consistent with the hypothesis, since even after six months in 1/3 SW, their prolactin cells adjust to FW by seven days. The value of following the morphological changes in the prolactin cells is illustrated by the parallel changes that occur in plasma sodium and other parameters in _Gillichthys_ and _Platichthys_ (Nagahama _et al_., 1973) and _Poecilia_ (Ball, 1969a; Ensor and Ball, 1972; Ingleton and Ball, 1972). However, some caution must be expressed about the design of experiments with teleosts. The current investigations suggest that the timing of physiological responses, as judged by morphological changes in the _eta_ cells, depends upon the duration of exposure to a particular environment. The nature of the experiment may be limited by the salinity tolerance of the experimental animals, since fishes such as _Anoptichthys_, _Carassius_, _Cichlasoma_ and _Xiphophorus_ can only tolerate dilute SW. The outcome of experiments with euryhaline fish may be determined by the ambient salt concentration prior to a transfer to FW, or whether the alterations in salinity are abrupt or gradual (Olivereau and Lemoine, 1972). Prolactin synthesis is activated in brackish water in _A. anguilla_ (Olivereau and Lemoine, 1972), _M. auratus_ (Olivereau, 1968), _P. latipinna_ (Olivereau and Ball, 1970) and _T. mossambica_ (Dharmamba and Nishioka, 1968). The introduction of fish by gradual changes in environmental salinity might serve to reduce stress effects produced by more abrupt ones. Another variable may be the developmental stage of the experimental fish (Leatherland,

1970a; Olivereau, 1968; Olivereau and Lemoine, 1972).

The responses of salmonid prolactin cells to decreasing ambient salinity are equivocal. Olivereau (1954, 1969) reported changes in *Oncorhynchus* and *Salmo* correlated with decreasing salinity, but others have not been able to distinguish changes in *eta* cell histology or ultrastructure during anadromous migration (Cook and van Overbeeke, 1969; McKeown and van Overbeeke, 1969; Robertson and Wexler, 1962; Woodman, 1939). Results of microspectrophotometric and immunochemical studies (McKeown and van Overbeeke, 1969, 1972) are not consistent with the pattern of prolactin cell activity observed in other teleosts. The differences in results obtained from salmonids by different investigators emphasizes the value of using laboratory animals. It is difficult to standardize the methods of collecting and handling animals in the field, especially when there is a reliance on commercial sources.

Fishes have been maintained in deionized or distilled water in attempts to exaggerate the influence of the environment on prolactin cell activity, and species differences have also been noted under these conditions. In *A. anguilla*, the *eta* cells exhibit cellular, nuclear and nucleolar atrophy, along with decreased RNA content (Olivereau, 1967a). These results are the reverse of those obtained from *A. jordani* (Mattheij and Sprangers, 1969) and *C. biocellatum* (Mattheij *et al.*, 1971). At the light microscopic level the cells from

C. auratus in deionized water do not appear to be different from those in FW, but at the ultrastructural level more Golgi bodies, mitochondria and endoplasmic reticulum are observed (Leatherland, 1972; Leatherland and Ensor, 1973).

Cell and nuclear hypertrophy, more pronounced nucleoli, increased cytoplasmic RNA and development of RER, mitochondria and Golgi elements generally indicate increased synthetic activity in cells. Prolactin cell hyperplasia, as well as hypertrophy, may contribute to the increased size of the pro-adenohypophysis in A. jordani (Mattheij and Sprangers, 1969) and F. heteroclitus (Emmart et al., 1966) in fresh or distilled water. The presence of mitotic figures has not been noted with the hypertrophy of the eta cells or RPD in X. maculatus (current investigations), O. latipes (Nagahama and Yamamoto, 1971), P. latipinna (Ball, 1969a, b), M. auratus (Olivereau, 1968), G. mirabilis, P. stellatus (Nagahama et al., 1973) and and T. mossambica (Dharmamba and Nishioka, 1968). It has been suggested that hyperplasia contributes to increased volume of the RPD in C. biocellatum (Mattheij et al., 1971), M. capito and M. cephalus (Blanc-Livni and Abraham, 1970) because of an inability to account for the increase on the basis of cellular hypertrophy alone, and yet cell division was not observed. The investigators did not consider the possible effects of hyperemia (Olivereau, 1968, 1969), intercellular fluid accumulation (Emmart et al., 1966), or increases in the volume of the non-granular chromophobes (Leatherland, 1970a)

on the change in the size of the pro-adenohypophysis. It is curious that with numerous observations of increased size of the RPD and increased activity of the prolactin cells in FW, reports of mitotic activity are scanty. The data and observations on the platyfish (experiments 1 & 2) yield no evidence of a diurnal rhythm, but Schreibman (1964) suggested that the inability to observe cell division might be attributed to a periodicity of the cell cycle that does not correspond with the time of fixation. Therefore, failure to observe mitotic figures does not preclude the genesis of prolactin cells by cell division.

F. Mechanisms of control of _eta_ cell physiology in the platyfish

Zambrano (1972) has suggested that there are two means by which prolactin cells are regulated in teleosts. _Eta_ cells may respond directly to osmotic pressure changes (the predominant one) or they may be controlled by the hypothalamus. Support for the concept of direct osmotic influence on the prolactin cell has been demonstrated with pituitary organ cultures from _Xiphophorus_ (Sage, 1968) and _Poecilia_ (Ingleton, Baker and Ball, quoted by Ball _et al._, 1972). Transplanted and _in situ eta_ cells respond similarly after a transfer from 1/3 SW to FW, in both _X. maculatus_ (experiment 6) and _P. latipinna_ (Ball _et al._, 1972). However, hormones from other glands may be exerting an effect upon the transplanted pituitary.

Hypophysectomized X. hellerii, X. maculatus (Schreibman and Kallman, 1964), Gambusia sp. (Chambolle, 1969) and P. formosa (Ball, Olivereau, Slicher and Kallman, 1965; Olivereau and Ball, 1964, 1966), which bear pituitary transplants survive indefinitely in FW. It is well documented that the product of the eta cells is primarily required for freshwater survival in poeciliid fishes. The cytological characteristics of eta cells in transplants, and associated physiological studies, support a concept that prolactin secretion continues in the grafts (Ball and Baker, 1969; Ball et al., 1972).

In X. maculatus, in dilute SW with or without an in situ pituitary, the cytology of transplanted eta cells suggests that their synthetic activity is under inhibitory control in the intact fish in 1/3 SW. The prolactin cells in grafts have prominent nucleoli and perinuclear halos. It should be recalled that in the RPD of intact platyfish, these signs of synthetic activity are reduced within 48 hours after entry into dilute dilute SW (experiment 2). In the case of the autografts it is logical to assume that there was a decrease in synthesis during the week in 1/3 SW before grafting, and that it increased during the two weeks afterward. Additional evidence for inhibitory control in saline media comes from increased nuclear areas in grafted eta cells in P. formosa (Olivereau and Ball, 1966) and P. latipinna (Ball et al., 1972). In contrast, autografted eta cells in C. auratus in dilute SW

do not appear to be different from those of intact fish (Leatherland and Ensor, 1973). This may represent a species difference since _C. auratus_ containing transplants were exposed to dilute SW for approximately the same time as _X. maculatus_ in the current studies.

Ultrastructural studies of the neurohypophysis, and the results of treatment with various drugs have suggested the means by which neural inhibition is manifested. _Eta_ cells are synthetically activated in SW _G. mirabilis_ when aminergic "B" fibers which predominate the neurohypophysis adjacent to the RPD are destroyed by intracisternal injections of 6-hydroxydopamine (Zambrano, Nishioka and Bern, 1972). Similarly, there is an activation and hypertrophy of _eta_ cells in _Mugil platanus_ in dilute SW after systemic injections of reserpine, a monamine oxidase inhibitor. Reserpine may also activate the _eta_ cells in _G. mirabilis_ (Sage, 1970), but it does not appear to have marked effects in _A. anguilla_ (Olivereau, 1971a). Olivereau (1971b) has suggested that the eel prolactin cell is primarily under stimulatory hypothalamic control from the pituitary.

The question arises as to whether transplanted prolactin cells are more active than _in situ_ cells in FW. This is difficult to evaluate because prolactin content in the _eta_ cells and in the circulation of fish bearing transplants has not been determined. Under the same experimental conditions (24 hours after a transfer from 1/3 SW to FW) larger nuclear areas suggest

that transplanted *eta* cells are "more active" than those *in situ* in *P. latipinna* (Ball *et al.*, 1972). Similar observations have been made on *A. anguilla* in FW (Olivereau, 1970; Olivereau and Dimovska, 1969). Stanley and O'Connell (1970) have suggested that prolactin is "hypersecreted" in autografted *Umbra limi* in FW, on the basis of an increase in the size and number of epidermal mucous cells in comparison to sham-operated controls. It is difficult to understand the state of prolactin cell activity in *Anguilla* because of an apparent contradiction in the interpretation of results from the cytological studies of transplants (Olivereau, 1970) and from studies by the same investigator on presumed physiological and target organ parameters (Olivereau, 1971a, b). Interpreting the activity of a specific hormone source on the basis of a *presumed* target parameter is hazardous, since the number of hormones produced by a graft and the effects of each have to be determined.

In spite of the difficulties in interpreting levels of hormone secretion, a correlation of *eta* cell morphology with data from other parameters may still be a useful approach. Epidermal mucous cells on scales from *X. maculatus* in 1/3 SW for six months are markedly reduced in number, but increase three days after a return to FW (Napoli, Holtzman and Schreibman, unpublished data). The validity of using mucous cells as an indicator of prolactin cell activity might gain support by determing whether there is a similar correlation with *eta* cell morphology in both grafted and *in situ* hypophyses.

High circulating levels of prolactin may regulate the release of this hormone by way of the hypothalamo-hypophysial axis (MacLeod, 1970) or by way of a direct (short-feedback) effect on the *eta* cell (Clemens and Meites, 1968; Kastin, Arimura, Schally and Miller, 1971; Spies and Clegg, 1971). Exogenous prolactin was used to study feedback inhibition in the platyfish (experiment 5). Chronic injections of doses equal to and five times those required for the maintenance of hypophysectomized *X. maculatus* in FW (Schreibman and Kallman, 1966) produced no detectable changes in the *eta* cells of FW animals after 14 days. Daily injections of prolactin into *Anguilla* for up to 50 days resulted in a reduction in nucleolar prominence, coupled with cytoplasmic vacuolation and degranulation (Ball and Olivereau, 1964; Olivereau, 1969). It is possible that 14 days is an inadequate duration to observe the appropriate changes in *Xiphophorus*. Tucker, Convey and Koprowski (1973) suggest that, in mammals, feedback control of release depends solely on long-term exposure to high chronic doses of prolactin, or that inhibition is exerted at the level of synthesis rather than release. However, in experiments where chronic doses are employed, the interpretation of results may be difficult because of stress phenomena that may be induced.

Exogenous prolactin was also administered to platyfish after 21 days in 1/3 SW to observe the effect on activation of the *eta* cell (experiment 5b). Six hours in FW was chosen as a minimum period to insure an increase in prolactin synthesis

in controls. Two injections of a high dose (50 µg) of ovine prolactin resulted in a slightly, but significantly, higher C.D. in fish returned to FW for six hours, when compared to vehicle-injected controls under the same conditions. The response is transient because there was no significant difference after 24 hours in FW. The small number of fish used in the experiment precludes drawing generalizations and the difference should be verified by repetition of the experiment.

The results of exogenous ovine prolactin treatment of X. maculatus may represent a case of species specificity in which an absence of a marked morphological response reflects refractoriness of platyfish cells to a foreign protein. This is suggested by the observation that salmon prolactin is more effective than ovine in elevating plasma osmolarity of the hypophysectomized goldfish (Donaldson et al., 1968). More definitive studies of exogenous prolactin will have to await the availability of purified teleostean hormone. The evidence, as described above, indicates that grafted eta cells continue to secrete prolactin. In the absence of purified fish prolactin, hypophysial transplants were used to study the possible effects of homologous prolactin on the in situ cell (experiment 6c).

The in situ cells of transplant-bearing intact platyfish in 1/3 SW do not appear to be synthetically active, but their

cytoplasmic chromophilia suggests an inhibition of hormone release. Furthermore, the recovery of synthetic activity in the *in situ eta* cells appears to be inhibited by the grafts after six hours in FW, agreeing with the results obtained from the administration of exogenous prolactin. The more pronounced effects of the grafts compared to those of ovine prolactin also provide support for the concept of species specificity of hormonal action. However, it must be reemphasized that other hormones from the grafts may be exerting an influence on the *in situ* prolactin cells. Presumptive adrenocorticotrophic, gonadotrophic and thyrotrophic cells have been identified in the platyfish grafts by their location, morphology and staining properties. Similarly, several cell types have been reported in grafts in *A. anguilla* (Olivereau and Dimovska, 1969), *C. auratus* (Leatherland and Ensor, 1973), *G. aculeatus* (Leatherland, 1970b), *P. formosa* (Olivereau and Ball, 1966) and *P. latipinna* (Ball *et al.*, 1972).

At this point the importance of delineating temporal changes in hormone producing cells in response to experimentally induced changes of the organism's physiology can be emphasized. The results of the transplant experiments serve to illustrate this concept. Hypophysial grafting in platyfish, using 21 days as an acclimation period in 1/3 SW, was carried out prior to an awareness of the effects of longer periods on intact fish. It has been noted that the structure and function of hypophysial transplants change with time (Ball *et al.*, 1972). It is

suggested that in future experiments platyfish bearing transplants should be adapted to FW for several months and then be subjected to short and long-term exposures to dilute SW, followed by a return to FW (e.g., experiments 3 & 4).

A recent experiment (McKeown, 1972) with 2-Br-α-ergocryptine-methane-sulfonate (CB-154, Sandoz) also underlines the importance of establishing temporal responses. McKeown (1972) suggested that CB-154 might be used to study the role of prolactin in the osmoregulatory physiology of teleosts, by specifically lowering circulating prolactin levels without having to resort to hypophysectomy. This compound may act directly on mammalian eta cells since it inhibits the release of prolactin in vitro (Pasteels, Danguy, Frérotte and Ectors, 1971). McKeown (1972) injected the drug into X. hellerii and P. latipinna which were returned to FW from seven days in dilute SW. It was suggested that the inhibition of prolactin release was reflected by increased eta cell chromophilia, reduced plasma sodium levels, and the inability of drug treated animals to survive in FW. Since the current series of investigations indicate that the initial rate of release exceeds that of synthesis in X. maculatus after a return to FW, it would be valuable to study the effect of CB-154 on prolactin release in fishes maintained in dilute SW for a longer period of time. Then the cytology of the eta cells in CB-154-treated and control fish could be compared within 48 hours after the transfer. One would expect to find

markedly degranulated cells in the controls, and accumulation of stainable secretory material in the experimental animals. These observations would support the interpretation of the results of the current studies.

SUMMARY AND CONCLUSIONS

The freshwater teleost, *Xiphophorus maculatus* (the platyfish), was placed into 1/3 SW for varying periods of time and then returned to FW. Prolactin-producing (*eta*) cells of the pituitary gland were studied by cytologic, cytometric and autoradiographic methods, and progressive changes in these parameters were noted during the adaptation of the fish to each environment. These methods permit one to distinguish between synthetic and secretory (release) activity. A high rate of synthetic activity is indicated by large cell size, pronounced nucleoli, perinuclear halos, and large numbers of silver grains over the prolactin cells. The degree of cytoplasmic chromophilia, when related to the observations of these variables, allows an evaluation of the release of secretory material during the adaptation of the platyfish to altered environmental salinity. The following conclusions were drawn.

Reduced synthetic activity was detected in the *eta* cell as early as 12 hours after a transfer into 1/3 SW. Degranulation and reduction in cell size continued for up to 36 additional hours. The morphology of the pro-adenohypophysis is similar in animals in dilute SW for 21 and 30 days, and is acheived in the initial 48 hours.

There is a critical period between 21 and 30 days in 1/3 SW in which the environment exerts a maximal effect on the

physiology of the prolactin cell. This effect is not manifested in the morphology of the cells, but is detected by differences in the rate of recovery of synthetic activity when platyfish are returned to FW.

Recovery of synthetic activity and accumulation of secretory material are evident by four hours after a return to FW from 21 days in 1/3 SW. In contrast, after 30 days in dilute SW, it takes 24 to 48 hours in FW before more prominent nucleoli and increased grain density are detected. Pronounced cytoplasmic chromophilia is not observed until 72 hours. These observations suggest that following prolonged exposure to 1/3 SW, the rate of release initially exceeds that of synthesis. Even when neonatal platyfish are placed into 1/3 SW for six months, the time of recovery is similar to that seen after 30 days.

Sexual dimorphism may be revealed in the prolactin cells of platyfish after six months in 1/3 SW. The _eta_ cells were larger and more chromophilic in sibling males than in their sisters. These observations will have to be confirmed because of the small number of animals used, but in a larger group of animals differences in growth patterns were also noted. The standard length of females in dilute SW was significantly less than that of those in FW, but there were no significant differences among the males. Because of the absence of differences in the size and morphology of the

somatotrophic cells and nuclei in FW and SW females, it is suggested that the growth-promoting effects of prolactin should be investigated in juvenile _Xiphophorus_.

Preliminary investigations on the control of prolactin synthesis and release were carried out with the use of exogenous prolactin and hypophysial transplants. Injections of ovine prolactin, up to five times the dose required to keep hypophysectomized platyfish alive in FW during a two week period, had no effect on the morphology and size of the _eta_ cells of intact FW fish. In fish returned to FW after 21 days in 1/3 SW, exogenous prolactin (administered 24 hours prior to, and at the time of transfer) had a transient effect on cytoplasmic chromophilia and cell size. Both parameters were less than those found in vehicle controls at six hours, but there were no differences by 24 hours.

Prolactin cells in ectopic pituitary transplants behave similarly, regardless of whether the host has been hypophysectomized or not. In fish in 1/3 SW, ectopic _eta_ cells are larger and have more prominent nucleoli than those that are _in situ_. This suggests that synthetic activity is greater when _eta_ cells are separated from central nervous system influence. It is therefore proposed that hormonal synthesis in the _in situ_ prolactin cells of fish in dilute SW is under inhibitory control from the hypothalamus. When platyfish bearing transplants are returned to FW from 21 days in 1/3 SW,

the grafted *eta* cells behave similarly to those of intact fish, suggesting that prolactin cells are directly responsive to osmotic changes.

After 21 days in 1/3 SW, the *in situ* prolactin cells of sham-hypophysectomized fish, bearing ectopic hypophysial transplants, display no signs of synthetic activity but are heavily granulated, possibly as a result of an inhibition of hormone release by the grafts. There are no changes in the *in situ* cells of fish returned to FW for six hours, intimating that the grafts are inhibiting synthesis as well as release. Although our knowledge of the variety of cells in the transplants and their hormonal activity requires further elucidation, it is suggested that homologous prolactin may exert feedback effects on *in situ eta* cells.

The results of the current series of investigations clearly demonstrate the need for caution when designing experiments which involve the study of hormonal responses in teleosts to environmental changes over a period of time. They also indicate that there must be a clear definition of the term "activity" that includes both synthetic and secretory phenomena. This can be achieved by the concomitant use and evaluation of several parameters.

ADDENDUM

Following the completion of this manuscript, Ball and Ingleton (1973) published a report on the response of prolactin cells in *Poecilia latipinna* to alterations in ambient salinity as judged by cytometric, cytologic and assay methods. Their conclusions and support are essentially similar to those presented in this thesis.

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Table 1. Outline of experimental designs.*

Exp. 1. FW ——————→ 1/3SW 21 days

Returned to FW

a	b	c
4 hr	4 hr	2 hr
12 hr	8 hr	4 hr
24 hr	12 hr	24 hr
	24 hr	

Exp. 2. FW → 1/3SW 2, 4, 12, 24, 48 hr & 30 days

Returned to FW 2, 4, 72 hr

Exp. 3. FW → 1/3SW → 21 days → a → FW 4, 24 hr; b → FW 4, 24 hr

FW → 30 days → a → FW 4, 24 hr; b → FW 4, 24, 48, 72 hr, 7 days

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Exp. 4. FW → 1/3SW 6 months → FW 48, 72, 96 hr, 7 days

Exp. 5a. FW 14 days: injected on alternate days 1-13; killed on day 14.

5b. FW ——→ 1/3SW 21 days → FW 6, 24 hr

Exp. 6. 1/3SW 21 days → FW 6 hr (exp. 5b: 6, 24 hr).

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*See materials and methods for additional details.

Table [illegible]. Effect of 21 days in one-third sea water.

Exp.	Strain, Sex & Age	Group	# of Fish	Cell Index*	Nuclear Index*	Cell Density*
1a.	163[illegible] male 10 months	Fw	[illegible]	0.5[illegible] ± .02'	0.32 ± .01'	1[illegible].2 ± 1.2'
		In 1/3Sw 21 days	[illegible]	0.3[illegible] ± .01	0.2[illegible] ± .01	21.1 ± 0.[illegible]
		Returned to Fw for				
		[illegible] hr	[illegible]	0.42 ± .01'	0.30 ± .004'	26.5 ± 2.0'
		12 hr	[illegible]	0.45 ± .01'	0.31 ± .003'	21.0 ± 0.3'
		24 hr	[illegible]	0.[illegible] ± .01'	0.30 ± .01	21.0 ± 1.6'
1b.	235[illegible] male 10 months	Fw	[illegible]	0.47 ± .04	0.29 ± .01	21.3 ± 0.3'
		In 1/3Sw 21 days	[illegible]	0.39 ± .01	0.2[illegible] ± .01	29.3 ± 0.5
		Returned to Fw for				
		[illegible] hr	[illegible]	0.46 ± .02'	0.27 ± .003	25.0 ± 1.1'
		[illegible] hr	[illegible]	0.43 ± .01'	0.27 ± .01	24.4 ± 1.2'
		12 hr	5	0.45 ± .02'	0.29 ± .003'	23.0 ± 1.1'
		24 hr	6	0.44 ± .02'	0.27 ± .01	22.8 ± 1.1'

Table [illegible]. continued

Exp.	Strain, Sex & Age	Group	# of Fish	Cell Index*	Nuclear Index*	Cell Density*
10.	1[illegible] male 11 months	FW	3	0.50 ± .03'	0.33 ± .01'	17.7 ± 0.[illegible]'
		In 1/3SW 21 days	[illegible]	0.39 ± .005	0.30 ± .005	27.[illegible] ± 0.9
		Returned to FW for				
		2 hr	[illegible]	0.40 ± .01	0.2[illegible] ± .01	29.3 ± 0.9
		4 hr	[illegible]	0.45 ± .01'	0.31 ± .01	23.0 ± 0.7'

*Mean ± SEM
'Significantly different from 1/3SW (p<0.05)

Table 3. Progressive changes in prolactin cells from fish in 1/3Sw.

Exp.	Strain, Sex & Age	Group	# of Fish	Cell Index*	Nuclear Index*	Cell Density*
2.	2356 male 11-15 months	Fw	4	0.53 ± .03	0.32 ± .01	19.[illegible] ± 1.3
		In 1/3Sw				
		2 hr	5	0.51 ± .02	0.32 ± .01	23.[illegible] ± 0.4
		4 hr	5	0.53 ± .02	0.32 ± .02	22.[illegible] ± 0.9
		12 hr	5	0.53 ± .03	0.33 ± .01	28.4 ± 2.4"
		24 hr	5	0.50 ± .02	0.32 ± .01	29.[illegible] ± 1.5"
		4[illegible] hr	4	0.44 ± .01"	0.30 ± .01	28.0 ± 2.0"
		30 days	4	0.45 ± .01"	0.33 ± .01	32.0 ± 1.[illegible]"
		Returned to Fw for				
		2 hr	[illegible]	0.42 ± .01	0.31 ± .01	33.3 ± 1.1
		4 hr	[illegible]	0.4[illegible] ± .01	0.32 ± .004	30.0 ± 1.[illegible]
		72 hr	3	0.43 ± .01	0.31 ± .01	31.7 ± 1.2

*Mean ± SEM
"Different from Fw controls ($p<0.05$)

Table [illegible]. Comparisons of 21 and 30 days in one-third sea water.

Exp.	Strain, Sex & Age	Group	# of Fish	Cell Index*	Nuclear Index*	Cell Density*
3a.	2[illegible] female 13 months	FW	[illegible]	0.5[illegible] ± .0[illegible]	0.[illegible] ± .01	1[illegible].[illegible] ± 0.6
		In 1/3SW 21 days	3	0.[illegible]1 ± .06"	0.2[illegible] ± .0[illegible]"	3[illegible].[illegible] ± [illegible]"
		Returned to FW for				
		[illegible] hr	3	0.4[illegible] ± .01	0.30 ± .01	26.[illegible] ± [illegible]
		24 hr	3	0.45 ± .02	0.29 ± .0[illegible]	26.0 ± [illegible].5'
		In 1/3SW 30 days	[illegible]	0.[illegible]3 ± .0[illegible]"	0.29 ± .01"	30.3 ± 2.4"
		Returned to FW for				
		[illegible] hr	3	0.45 ± .03	0.30 ± .01	29.3 ± 1.5
		24 hr	3	0.42 ± .02	0.32 ± .01	30.0 ± 1.0

Table 4. continued

Exp.	Strain, Sex & Age	Group	# of Fish	Cell Index*	Nuclear Index*	Cell Density*
3b.	163B female 2 years	FW	3	0.51 ± .02	0.31 ± .0[illegible]	22.3 ± 2.4
		In 1/3SW 21 days	[illegible]	0.39 ± .01"	0.28 ± .02"	33.[illegible] ± 0.[illegible]"
		Returned to FW for				
		[illegible] hr	4	0.45 ± .02'	0.31 ± .0[illegible]	27.0 ± 1.7'
		2[illegible] hr	4	0.[illegible] ± .01'	0.31 ± .0[illegible]	25.[illegible] ± 0.6'
		In 1/3SW 30 days	[illegible]	0.[illegible] ± .01"	0.29 ± .00[illegible]	32.9 ± 1.9"
		Returned to FW for				
		[illegible] hr	3	0.41 ± .0[illegible]	0.29 ± .01	33.0 ± 1.2
		24 hr	3	0.3[illegible] ± .01	0.31 ± .00[illegible]	29.0 ± 3.1
		48 hr	4	0.42 ± .01	0.31 ± .01'	26.5 ± 1.7'
		72 hr	4	0.45 ± .01'	0.32 ± .004'	25.5 ± 1.4'
		7 days	4	0.46 ± .01'	0.30 ± .01	20.0 ± 1.0'

*Mean ± SEM
'Different from 1/3SW controls ($p<0.05$)
"Different from FW controls ($p<0.05$)

Table 5. Effects of maintaining neonatal platyfish in one-third sea water for six months.

Exp.	Strain sex & age	Group	# of Fish	Cell Index*	Nuclear Index*	Cell Density*
I.	16[illegible] male + female 6 months	FW	1[illegible]	0.52 ± .01	0.32 ± .01	18.3 ± 0.5
		In 1/3SW 6 months	[illegible]	0.35 ± .01"	0.29 ± .01"	30.7 ± 1.4"
		Returned to FW for				
		48 hr	[illegible]	0.37 ± .01	0.2[illegible] ± .003	26.3 ± 2.3'
		72 hr	5	0.46 ± .01'	0.33 ± .01 '	22.2 ± 1.0'
		96 hr	5	0.46 ± .01'	0.30 ± .01	20.0 ± 0.4'
		7 days	3	0.4[illegible] ± .02'	0.30 ± .01	19.3 ± 0.3'
	females	FW	5	0.52 ± .02	0.32 ± .01	18.0 ± 0.9
		In 1/3SW 6 months	4	0.34 ± .01"	0.29 ± .01"	32.5 ± 1.2"
		Returned to FW for				
		48 hr	2	0.36 ± .02	0.30 ± [illegible]	25.5 ± 5.5'
		72 hr	3	0.46 ± .01'	0.34 ± .02'	22.7 ± 1.9'
		96 hr	3	0.46 ± .02'	0.29 ± .01	20.0 ± 0.6'
		7 days	3	0.48 ± .02'	0.30 ± .01	19.3 ± 0.3'

Table 6. (continued)

males	Fw	5	0.51 ± .01	0.32 ± .01	1[illegible].6 ± 0.7
	In 1/3Sw 6 months	[illegible]	0.39 ± .02"	0.2[illegible] ± .004"	27.0 ± 1.0"
	Returned to Fw for				
	48 hr	[illegible]	0.39 ± [illegible]	0.29 ± [illegible]'	27.[illegible] ± [illegible]
	72 hr	[illegible]	0.[illegible]5 ± .01'	[illegible].3[illegible] ± .01'	21.[illegible] ± 0.[illegible]'
	96 hr	[illegible]	0.[illegible]6 ± .01'	0.31 ± .01'	2[illegible].0 ± 1.0'

*Mean ± SE

'Different from 1/3Sw controls ($p<0.05$)

"Different from Fw controls ($p<0.05$)

Table [illegible]. Effects of exogenous ovine prolactin.

Exp.	Strain, Sex & Age	Group	No. of Fish	Cell Index*	Nuclear Index*	Cell Density*
5a.	163F male 10 months	FW saline inj.	[illegible]	0.[illegible] ± .01	0.33 ± .01	[illegible] ± [illegible]
		PRL 10 ug	[illegible]	0.52 ± .02	0.32 ± .004	1[illegible].3 ± 1.4
		50 ug	[illegible]	0.[illegible] ± .01	0.32 ± .01	19.0 ± 1.2
5b.	163F male 10 months	Saline inj. FW	[illegible]	0.51 ± .01	0.33 ± .01	19.[illegible] ± 0.5
		In 1/3 SW 21 days	5	0.40 ± .01	0.2[illegible] ± .01	26.[illegible] ± 0.8
		Returned to FW for 6 hr	[illegible]	0.[illegible] ± .00[illegible]	0.33 ± .003	20.[illegible] ± 1.0
		24 hr	3	0.[illegible] ± .01	0.33 ± .01	24.[illegible] ± 1.6
		PRL 100 ug 6 hr	5	0.[illegible] ± .01	0.32 ± .01	22.6 ± 0.[illegible]
		24 hr	5	0.[illegible] ± .01	0.32 ± .004	23.[illegible] ± 0.6

*Mean ± SEM

Table 7. Effects of pituitary transplantation.

[illegible]	[illegible]	Group	[illegible]	[illegible] Cell Index*	Nuclear Index*	[illegible]*
6a.	163B female 11-12 months	<u>Sham-hypophysectomized & sham-transplanted</u>				
		1/3 SW 21 days	2	0.39 ± .01	0.29 ± .01	27.5 ± 1.5
		FW 6 hr	2	0.45 ± .07	0.35 ± .02	23.5 ± 0.4'
		<u>Autotransplanted</u>				
		1/3 SW 21 days	3	0.41 ± .01	0.34 ± .02	25.7 ± 0.7
		FW 6 hr	4	0.46 ± .02	0.33 ± .01	19.1 ± 1.2'
		FW 24 hr	4	0.45 ± .02	0.33 ± .01	18.8 ± 0.6'
		<u>Sham-hypophysectomized & isotransplanted</u>		<u>in situ</u> pituitaries		
		1/3 SW 21 days	3	0.43 ± .01	0.31 ± .01	26.7 ± 1.7
		FW 6 hr	3	0.43 ± .03	0.30 ± .01	26.0 ± 1.7
				isotransplants		
		1/3 SW 21 days	3	0.41 ± .04	0.31 ± .02	26.3 ± 1.2
		FW 6 hr	3	0.48 ± .02'	0.34 ± .01	20.7 ± 1.8'

*Mean ± SEM
'Different from 1/3 SW controls ($p<0.05$)

[illegible]

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	–	–	–	–	–
[illegible]	–	–	–	–	–
[illegible]	[illegible]		–		
[illegible]	–	–			
[illegible]	–	–	–		
[illegible]	–	–	–		–
[illegible]	–				
[illegible]	–	–	–		
[illegible]	–	–	–		–
[illegible]	–	–	–		
[illegible]	–	–	–	–	
[illegible]	–	[illegible]	–		
[illegible]	–	–	–		
[illegible]	–	–			
[illegible]	–	–	–		–
[illegible]	–		–		
[illegible]	–	[illegible]	–	–	
[illegible]	–	–	–	–	
[illegible]	–		–	–	

[illegible]

[illegible]

[illegible]	Particle size	[illegible]	[illegible]	References
–	–			3,10,1[illegible]-2[illegible]
–				1[illegible],2[illegible]
–				[illegible]
–		–	–	[illegible]
				[illegible]
–				[illegible]
–		–	–	11, [illegible]
	–	–	–	[illegible]
–				[illegible]
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–	–		–	[illegible]
–	–	–		[illegible]
		–	–	[illegible]
–				[illegible]
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–		–		[illegible]
–	–	–	–	[illegible]
–	–			
–				[illegible]

[illegible]

Fig. 1. Sagittal section of a pituitary from a FW fish (Experiment 1c): P, pro-adenohypophysis; ME, meso-adenohypophysis; MT, meta-adenohypophysis; N, neurohypophysis. Masson's trichrome. 220x.

Fig. 2. Enlargement of the dorsal region of the pro-adenohypophysis in fig. 1 showing eta cells (dark cytoplasm) interspersed with degranulated and chromophobic cells; A, posterior wedge of adrenocorticotrophic cells. 560x.

Fig. 3. a. Prolactin cells around a sinusoid (S) in a FW fish (Experiment 1b); b. Autoradiograph of an adjacent section (film exposed for 8 weeks). 2200x.

Fig. 4. a. Prolactin cells from a fish in 1/3 SW for 21 days (Experiment 1b); b. Autoradiograph of an adjacent section (12 weeks exposure). Compare the labeling, morphology and size of the cells with those shown at the same magnification in figs. 3a, b.

Fig. 5. a. Prolactin cells from a fish in FW for 4 hours after 21 days in 1/3 SW in experiment 1b; b. Autoradiograph of an adjacent section (8 weeks exposure). Note that cytoplasmic chromophilia and radioactive labeling is greater than that of the cells in figs. 4a, b. Although the cells appear to be the same size the magnification of fig. 5a is almost half that of fig. 4a. 1460x.

Fig. 6. Prolactin cells from a fish in 1/3 SW for 12 hours (Experiment 2). Note the cytoplasmic chromophilia in (a) and the low grain density in (b), an autoradiograph of an adjacent section (12 weeks exposure). 1380x.

Fig. 7. a. Prolactin cells from a fish in 1/3 SW for 48 hours (Experiment 2). Compare with the cells in figs. 4a, 6a; b. Autoradiograph of an adjacent section (12 weeks exposure). 1260x.

Fig. 8. a. Prolactin cells from a fish in FW for 72 hours after 30 days in 1/3 SW; b. Autoradiograph of an adjacent section (8 weeks exposure). 1260x.

Fig. 9. a. Prolactin cells from a female that was reared from birth in 1/3 SW for 6 months (Experiment 4); b. Autoradiograph of an adjacent section (12 weeks exposure). 1910x.

Fig. 10. a. Prolactin cells from a male under the same conditions as the previous figure. Note the differences in nuclear and cytoplasmic morphology; b. Autoradiograph of an adjacent section (12 weeks exposure). 1980x.

Fig. 11. Prolactin cells from a female in FW for 48 hours after 6 months in 1/3 SW (Experiment 4). Note the large nucleoli and compare with fig. 9a. 2010x.

Fig. 12. Autoradiograph of a pituitary from a FW fish in experiment 4 (8 weeks exposure). 160x.

Fig. 13. Autoradiograph of a pituitary from a fish in FW for 48 hours after 6 months in 1/3 SW (Experiment 4, 8 weeks exposure). Note the extremely marked grain density over the pro-adenohypophysis. 160x.

Fig. 14. Transplanted prolactin cells in a fish in 1/3 SW for 21 days (Experiment 6c). Note the variation in extent of cytoplasmic granulation.

Fig. 15. *In situ* prolactin cells from the same animal as those in fig. 14. Note the cytoplasmic chromophilia and compare with fig. 4a, 7a. 1850x.

Fig. 16. Transplanted prolactin cells in a fish returned to FW for 4 hours after 21 days in 1/3 SW (Experiment 6c).

Fig. 17. *In situ* prolactin cells from the same animal as those in fig. 16. Note the similarity to fig. 15. 1570x.

Fig. 18. Effects of 21 days in 1/3 SW upon prolactin cell size when 163B males are returned to FW (Experiment 1a).

Fig. 19. Effects of 21 days in 1/3 SW upon prolactin cell

size when 2356 males are returned to FW (Experiment 1b).

Fig. 20. Effects of raising newborn platyfish in 1/3 SW upon prolactin cell size (Experiment 4).

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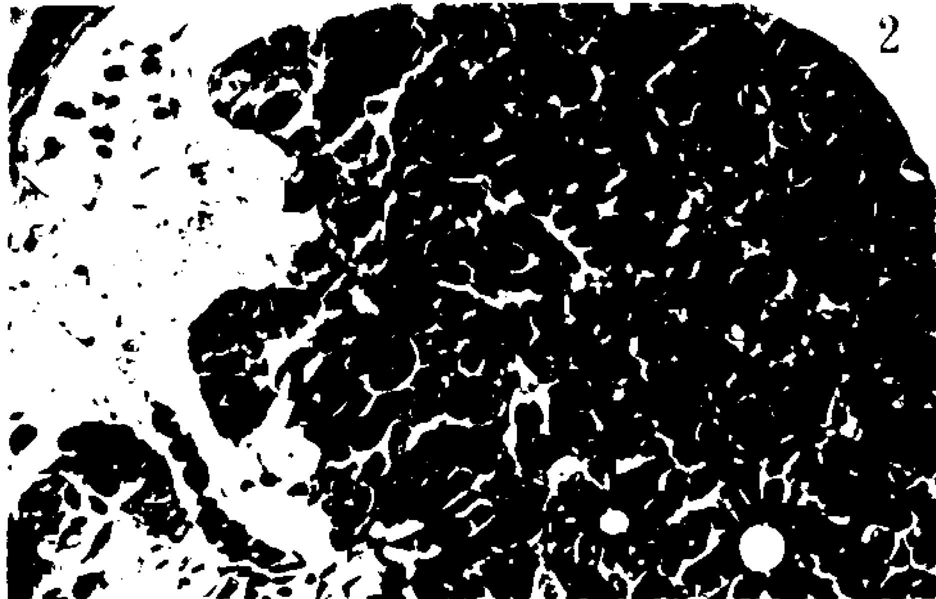
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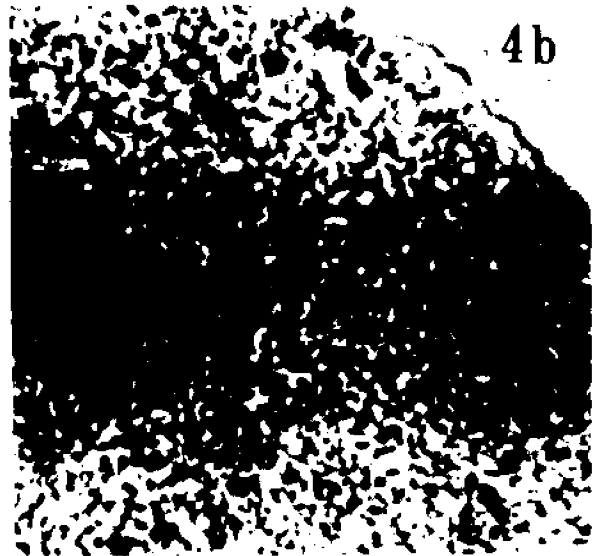
3 a
S

S

4 a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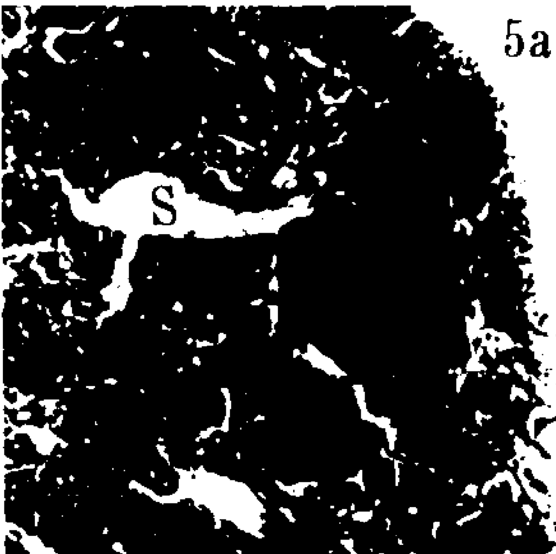
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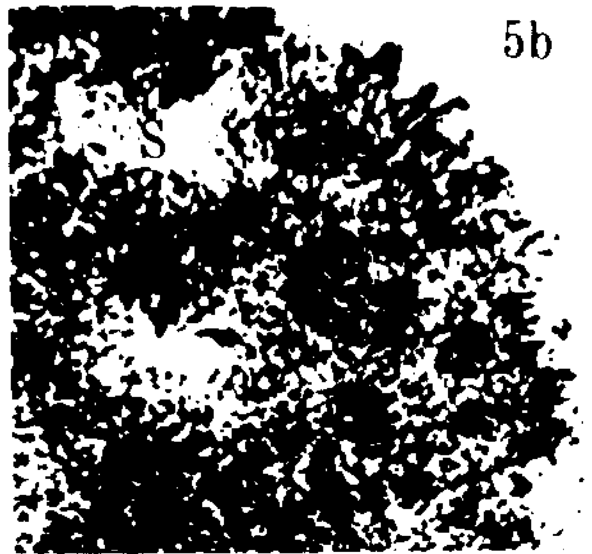
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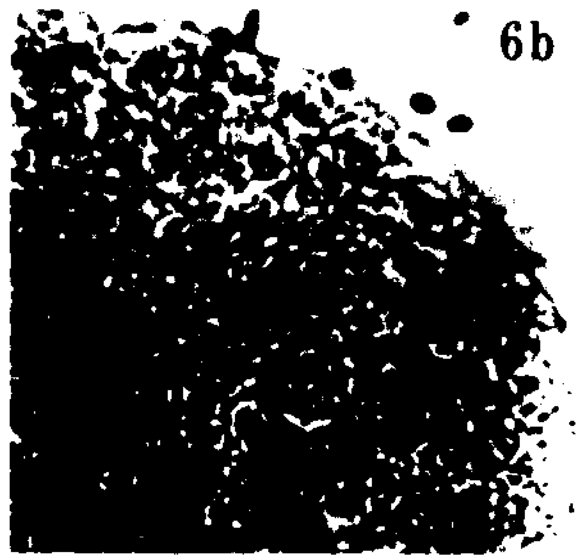
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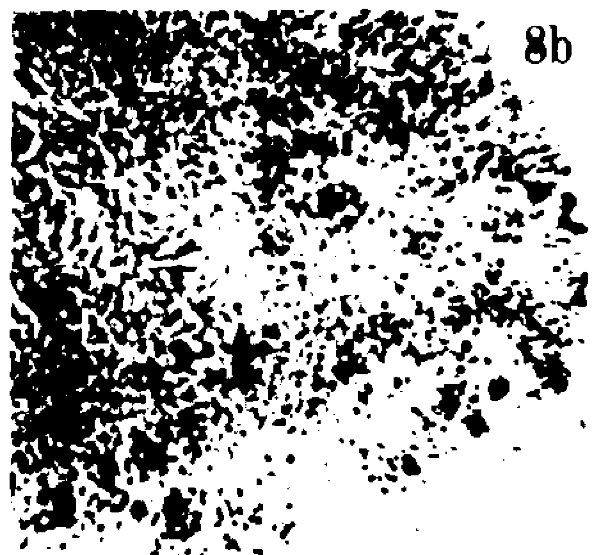
7a

7b

8a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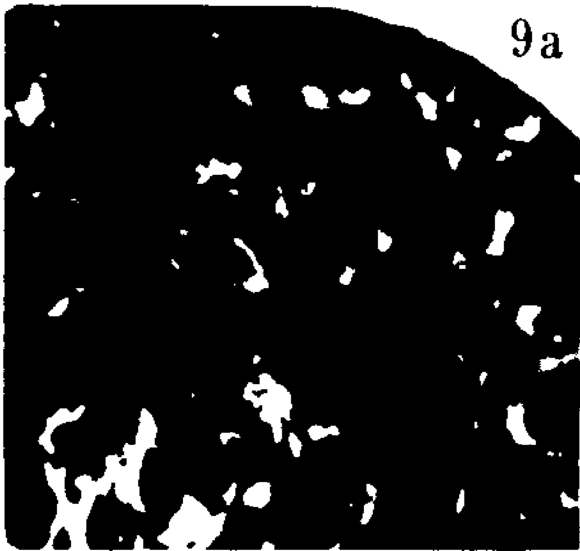
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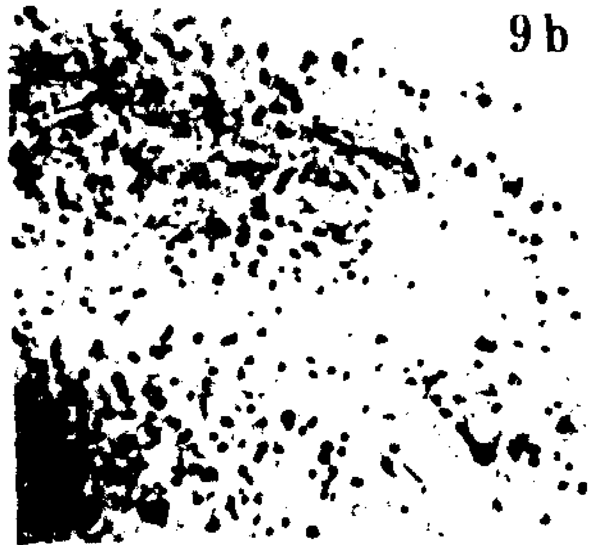
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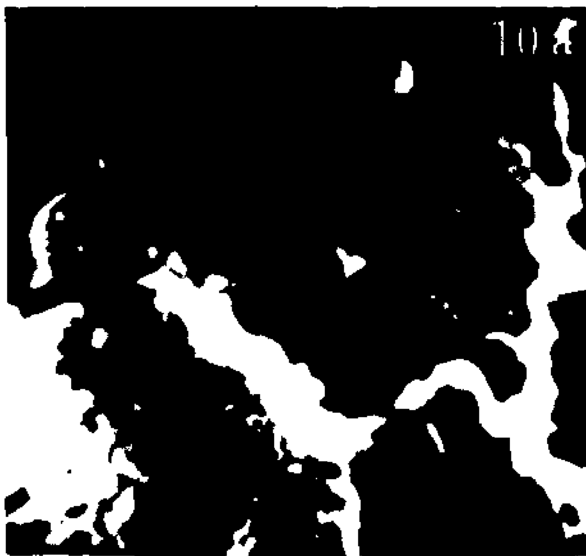
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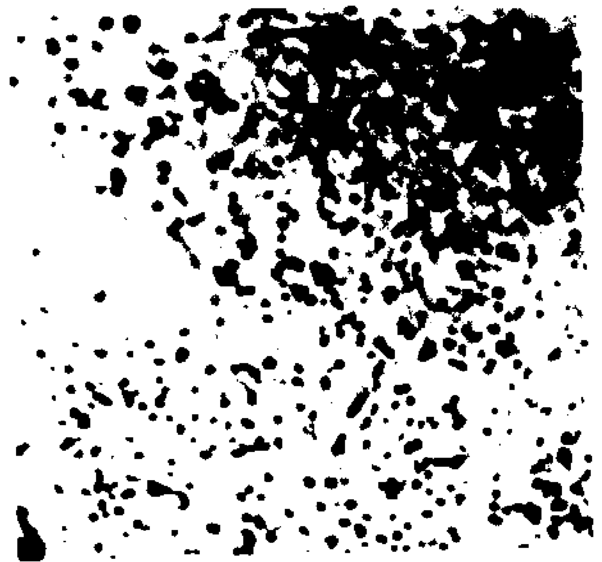

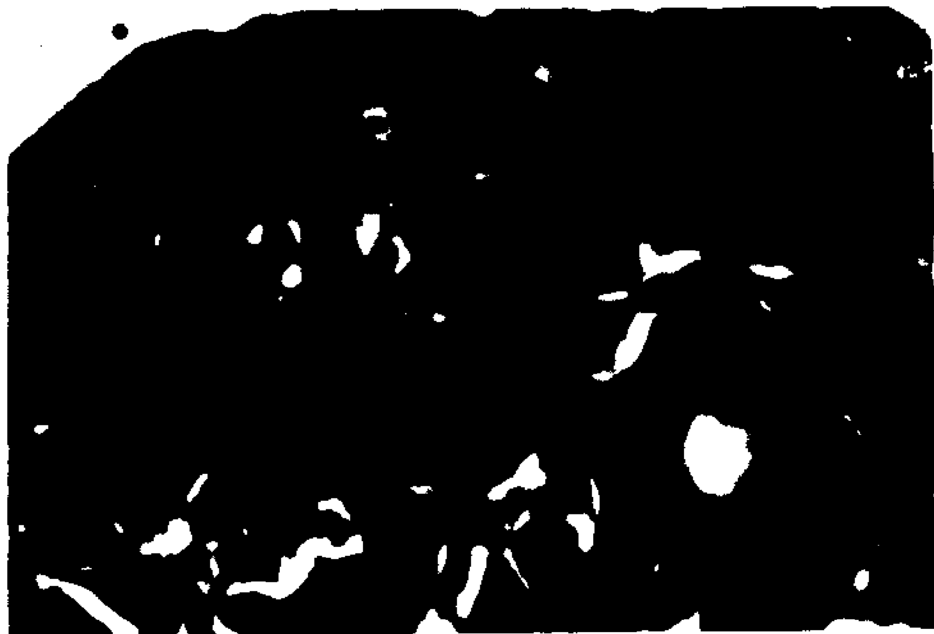

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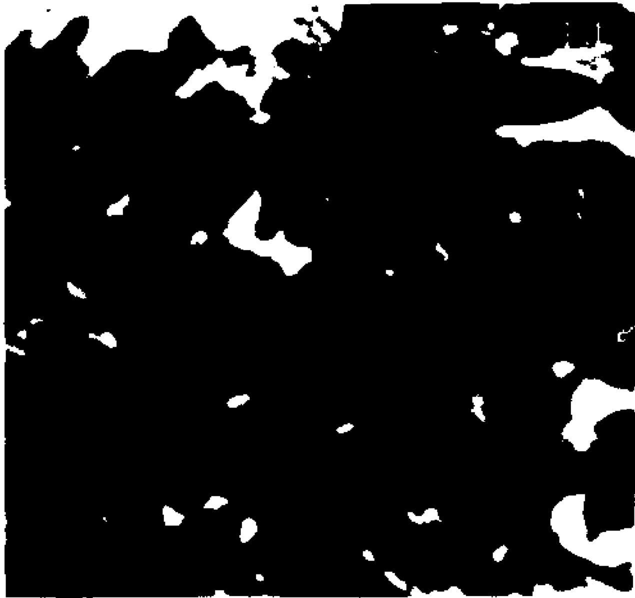

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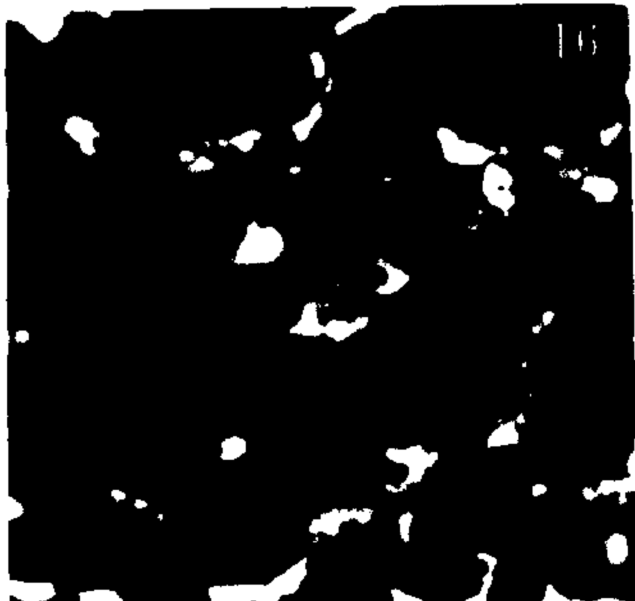
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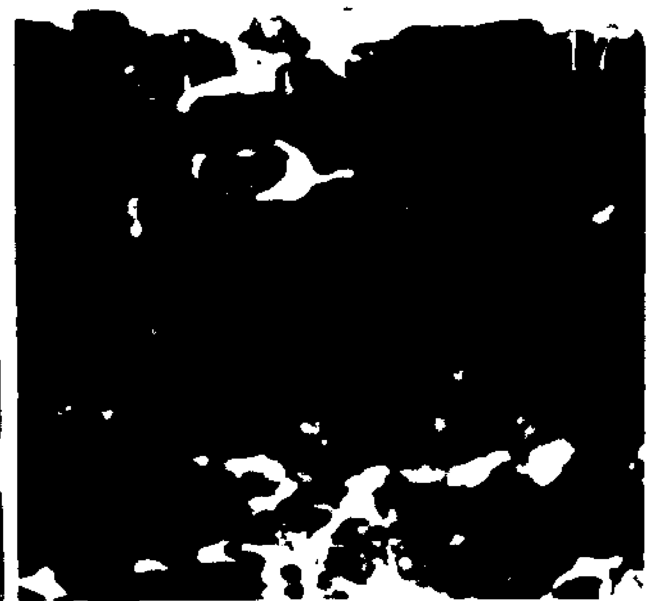
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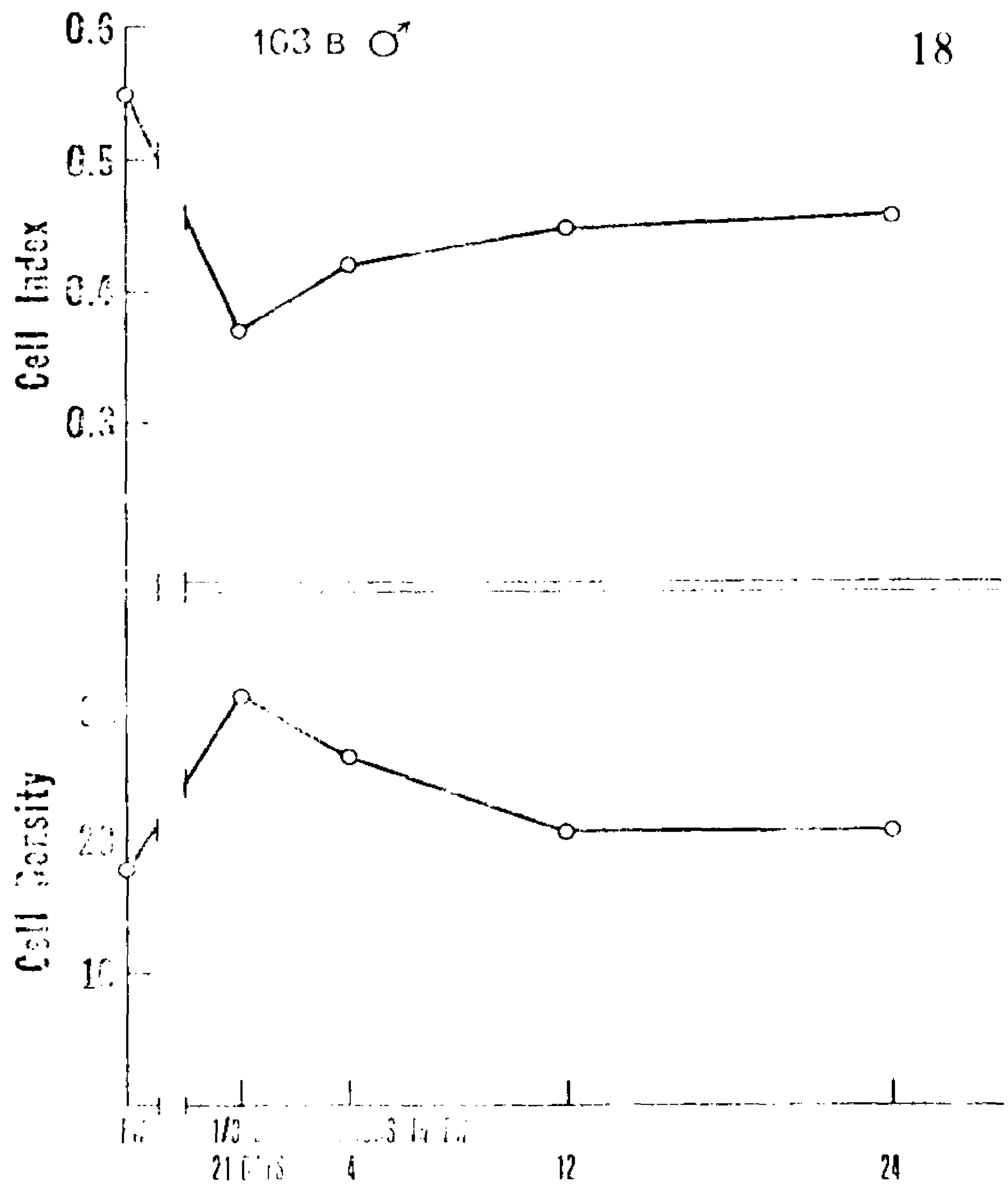

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0.4
0.3
Cell Density
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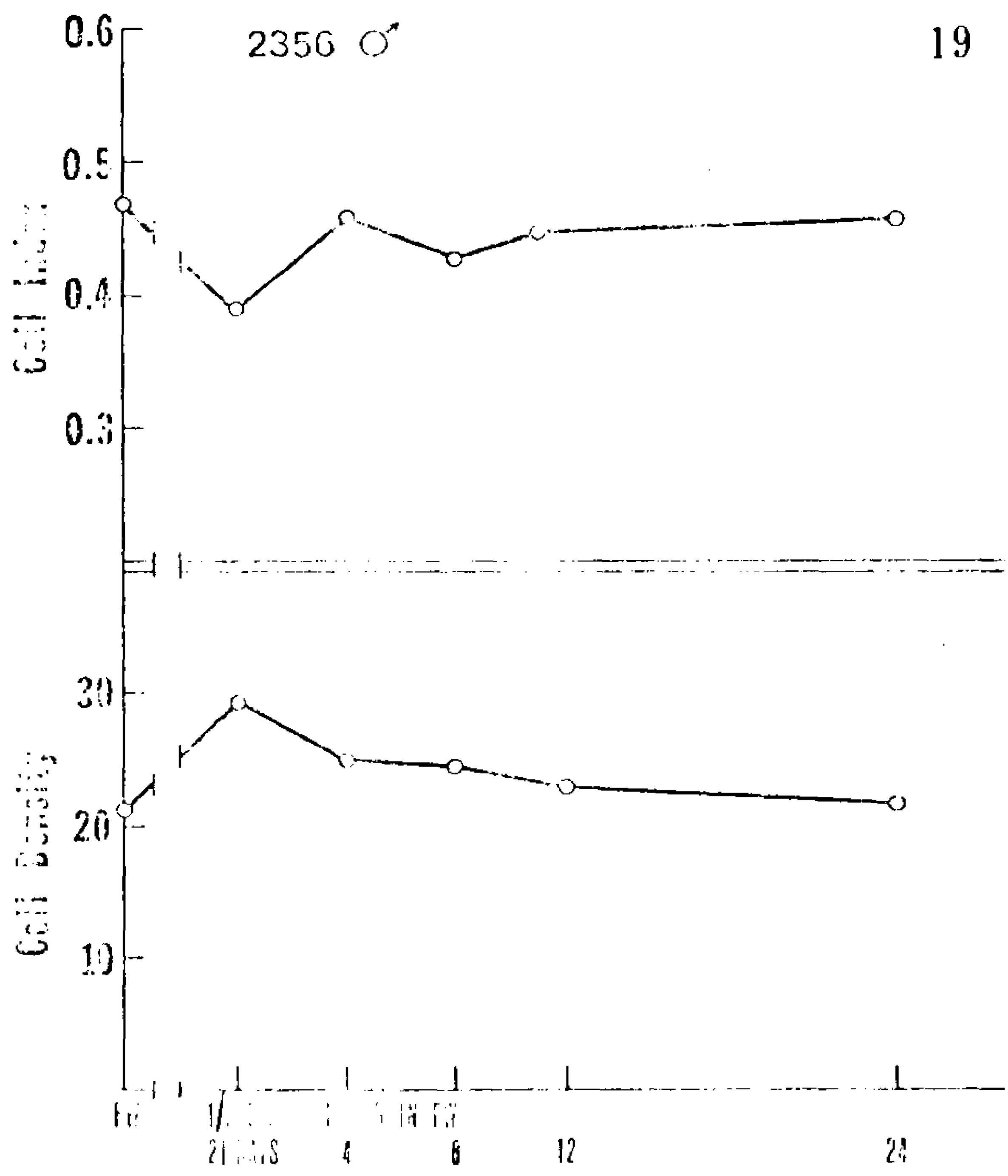
10
1/3
4
12
24



2356 ♂
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0.5
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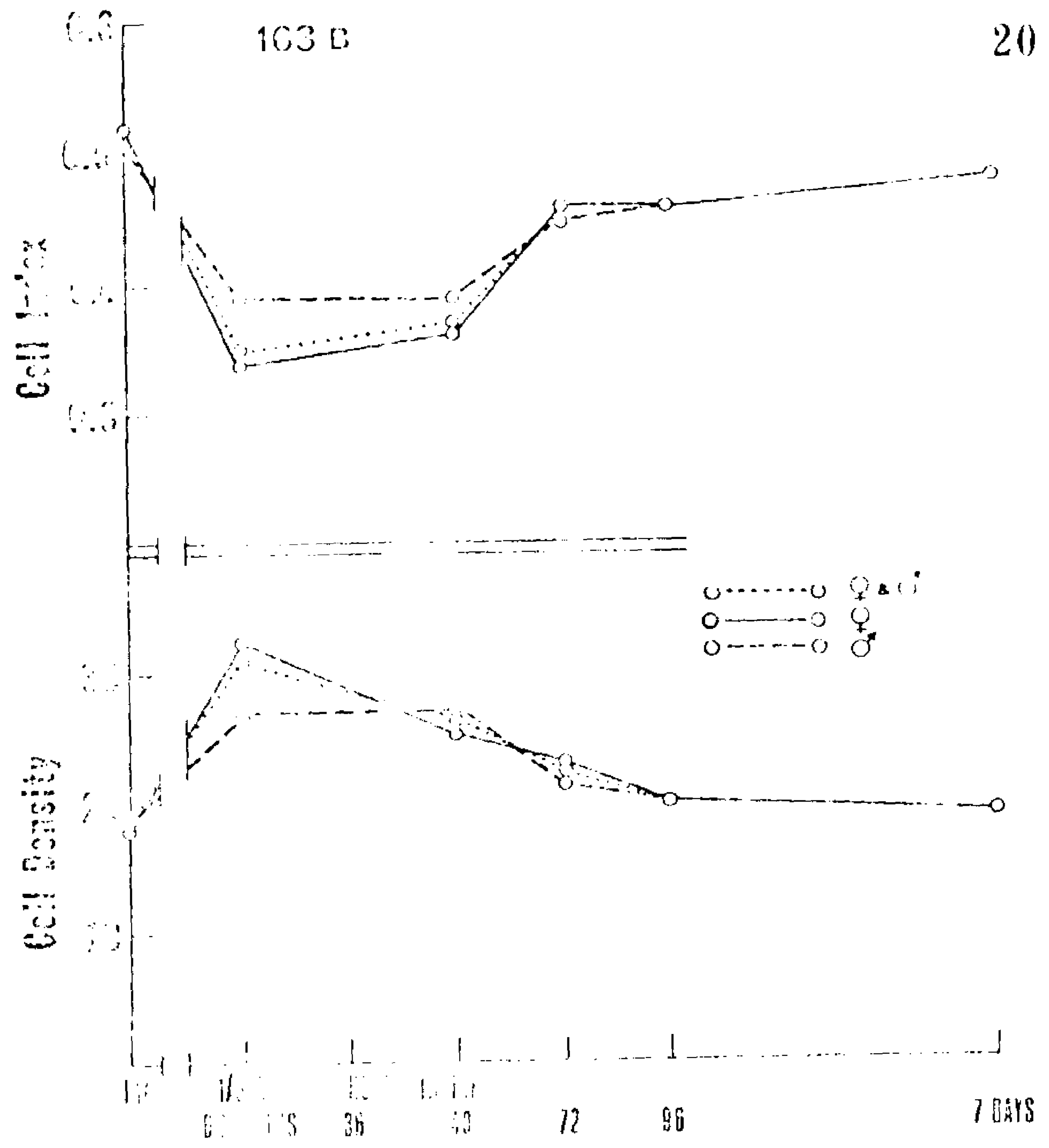
0.3
30
20
10
4
6
12
24


163 B
20
Cell Density
♀ & ♂
♀
♂
36
72
96
7 DAYS