A HISTOMETRIC INVESTIGATION OF THE ACTIVITY OF THE

PITUITARY-INTERRENAL AXIS IN JUVENILE COHO

SALMON, ONCORHYNCHUS KISUTCH WALBAUM

by

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ABSTRACT

The activity of the pituitary-interrenal axis in juvenile coho salmon (Oncorhynchus kisutch) and the relationship of this activity to the numbers of circulating leucocytes has been studied, using histological and histometric techniques. To this end, the effects of injections of mammalian ACTH, of cortisol acetate and of dexamethasone on the interrenal tissue, on the epsilon cells in the pituitary gland, and on the differential leucocyte counts were investigated. In addition, the activity of the pituitary-interrenal axis and the related changes in leucocyte counts were determined throughout their year of stream residence. Further, the response of this axis, along with corresponding hematological changes to environmental alterations in the laboratory, were studied.

Injections of ACTH resulted in a dosage-related stimulation of the interrenal tissue of coho salmon fry. On the other hand, injections of cortisol acetate and of dexamethasone produced a marked atrophy of the inter-renal tissue. All dosages of either ACTH or cortisol acetate decreased the mean nuclear diameters of epsilon cells, and resulted in their degranulation. In addition, a decrease in numbers of circulating small lymphocytes and thrombocytes resulted from administration of all dosages of ACTH, cortisol acetate or dexamethasone. It is proposed that the interrenal tissue of coho fry is capable of marked variations in activity, that this tissue is under pituitary control, and that a negative-feedback mechanism operates between the interrenal and the pituitary gland. Additionally, changes in pituitary-adrenocortical activity are reflected in characteristic alterations in numbers of certain circulating leucocytes.

The pituitary-interrenal axis of juvenile coho salmon in their natural habitat is inactive, from the time of emergence in spring, and through summer and early fall, compared with the winter and spring samples

of yearling coho. In addition, numbers of circulating small lymphocytes were decreased in the winter and spring samples of yearling coho compared with summer and autumn samples. It is suggested that the increased activity of the pituitary-interrenal axis along with changes in leucocyte counts observed in the winter sample of juvenile coho salmon are related to cold-temperature acclimation. Furthermore, it is proposed that the increased pituitary-adrenocortical activity noted in the latest of the three spring samples of yearling coho salmon is associated with the transformation from parr to smolt.

The interrenal tissue of juvenile coho salmon maintained in continuous darkness, or exposed to a continuously flashing light for varying numbers of days, was generally more active than that of corresponding control fish subjected to a twelve hour photoperiod. Additionally, small-lymphocyte and thrombocyte counts for darkness-maintained and flash-exposed fish were lower than values for corresponding control samples. On the other hand, no consistent differences in activity of the interrenal tissue were found when fish maintained in continuous light for varying numbers of days and corresponding control fish were compared. It was observed that the pituitary-interrenal axis of juvenile coho salmon was initially stimulated following transfer of the fish from holding tanks to an altered environment. In addition, the activity of the interrenal tissue was increased by exposure of these fish to cold water temperatures; this increased interrenal activity was accompanied by a lymphopenia. It is suggested that the pituitary-interrenal axis of juvenile coho salmon is involved in cold-temperature acclimation.

It is concluded that the pituitary-interrenal axis of juvenile coho salmon undergoes marked fluctuations in activity as a result of environmental alterations within the laboratory. Furthermore, an increase in pituitary-adrenocortical activity during acclimatization is characteristically reflected in a decrease in number of circulating small lymphocytes.

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GENERAL INTRODUCTION

In man and other mammals, increased secretory activity of the adrenal cortex occurs in response to such diverse stimuli as sudden temperature changes, strange environments, crowding, handling, restraint, sound, intense light, oxygen deficiency, radiation, infectious diseases and numerous other adverse stimuli (Selye, 1950; Gorbman and Bern, 1962; and Turner, 1966). The functional status of the adrenal cortex is regulated by secretions of adrenocorticotrophin (ACTH) from cells located in the pars distalis of the pituitary gland, and it is apparent that ACTH secretion is affected by such stimuli.

Although numerous studies with teleost fish recently have involved an examination of interactions between the interrenal (adrenocortical) tissue and the pituitary gland, and several investigations have attempted to ascertain the physiological role of the adrenal cortical secretions, the activity of the pituitary-interrenal axis of lower vertebrates exposed to varying environmental conditions has received little attention. It is assumed that the pituitary-adrenal system of lower vertebrates responds to tissue damage or environmental extremes in much the same manner as it does in the mammals (Hoar, 1966), although little evidence is available to substantiate this. Some support for this assumption is provided by the results of exposure of fish to a variety of conditions:- the spawning migration in Pacific salmon (Robertson and Wexler, 1959), temperature extremes in the goldfish (Mahon, Hoar and Tabata, 1962), the transfer of goldfish from one aquarium to another (Chavin, 1964), and holding rainbow trout in an aquarium with the water level markedly lowered (Donaldson and McBride, 1967).

The relationship between the activity of the pituitary-adrenocortical axis and leucocytes is interesting. During stimulation of the adrenal cortex of mammals, the numbers of circulating leucocytes is altered. Characteristically, increased adrenal cortical activity in mammals is accompanied by a decrease in numbers of circulating small lymphocytes and eosinophils, along with involution of the thymus, spleen and lymph nodes (Dougherty and White, 1944; Selye, 1950). In teleost fishes, effects of injections of mammalian ACTH and corticosteroids on blood cell counts have been noted (Weinreb, 1958; Slicher, 1961; Slicher and Ball, 1962); however, no studies correlating activity of the pituitary-interrenal axis of fish exposed to differing environmental conditions with changes in numbers of circulating blood cell types have been undertaken. Therefore, it was thought that it would be worth-while to determine whether or not a lower vertebrate would show similar changes when exposed to environmental alterations.

The experimental animal selected for this investigation was the juvenile coho salmon, <u>Oncorhynchus</u> <u>kisutch</u>. This salmonid, considered a primitive teleost, resides in local streams for a period of one year from time of emergence from the gravel; this is followed by seaward migration. The juvenile coho salmon was chosen for its abundance in nearby streams, its sexual immaturity, and also for its reported hardy characteristics when maintained under laboratory conditions.

With the aforementioned considerations in mind, the purposes of this research were:-

1. To establish whether or not the pituitary-interrenal axis functions (or is capable of functioning) in coho salmon fry, and the relationship of this axis to the circulating blood cells. Injections of

ACTH, cortisol and dexamethasone were carried out to investigate the activity of the system.

2. To determine the activity of this axis and related hematological changes throughout the freshwater life of juvenile coho salmon, by sampling fish seasonally from their natural environment.

3. To determine the sensitivity of the pituitary-interrenal axis and related hematological changes to environmental alterations within the laboratory (continuous darkness, continuous light, flashing light and temperature variations).

SECTION I: PRESENCE OF A PITUITARY-INTERRENAL AXIS AND ITS RELATIONSHIP
             TO THE CIRCULATING LEUCOCYTES

INTRODUCTION

The interrenal gland of teleost fish is, in most species, a somewhat diffuse tissue lying along the posterior cardinal veins or their branches within the anterior or "head" kidney (van Overbeeke, 1960; Nandi, 1962). On the basis of its embryology, histology and biochemistry, numerous studies have established that the interrenal gland is homologous to the mammalian adrenal cortex (Pickford and Atz, 1957; Chester Jones and Phillips, 1960; Bern and Nandi, 1964). Valuable evidence favoring this homology involves histochemical (Chavin and Kovacevic, 1961; Chavin, 1966) and fine-structural (Yamamoto and Onozato, 1965; Ogawa, 1967) studies of interrenal tissue, and in vitro incubation studies with labelled precursors of steroid synthesis (Phillips and Mulrow, 1959; Nandi and Bern, 1960; Butler, 1965; Arai et al., 1969). Incubation of teleostean head kidneys, as well as direct extraction of this kidney region (Fontaine and Leloup-Hatey, 1959), have demonstrated that the portion of the kidney containing interrenal cells is a source of corticosteroids. Adrenocorticosteroids have been clearly identified in plasma of teleost fishes (Bondy et al., 1957; Chester Jones and Phillips, 1960; Nandi and Bern, 1965; Chester Jones et al., 1970). Cortisol was shown to be a major corticosteroid in the plasma of salmonids (Hane and Robertson, 1959; Schmidt and Idler, 1962; Donaldson et al., 1968), and plasma cortisol levels have been determined as an index of interrenal activity (Fagerlund, 1967; Donaldson and McBride, 1967; Fagerlund et al., 1968; Fagerlund and McBride, 1969).

A number of recent investigations provide evidence for a pituitary regulation of the interrenal tissue in teleosts. Following hypophysectomy, atrophy of interrenal tissue occurs in Anguilla anguilla (Fontaine and Hatey, 1953; Carassius auratus (Chavin and Kovacevic, 1961); Tilapia mossambica (Basu et al., 1965); Couesius plumbeus (van Overbeeke and Ahsan, 1966) and in Salmo gairdnerii (Donaldson and McBride, 1967). Rasquin (1951) first showed that mammalian ACTH elicits hypertrophy of the interrenal tissue in Astyanax mexicanus. This observation was later confirmed in several other species: Anguilla anguilla (Fontaine and Hatey, 1953), Carassius auratus (Chavin, 1956), Umbra krameri (Krauter, 1958), Tilapia mossambica (Basu et al., 1965) and in Oncorhynchus nerka (Fagerlund et al., 1968). Evidence for the presence of a corticotrophic hormone in the teleost pituitary has been provided by observations of interrenal hypertrophy following implantation of teleost pituitaries into intact or hypophysectomized Astyanax mexicanus (Rasquin, 1951) and Carassius auratus (Chavin, 1956). Similarly, van Overbeeke and Ahsan (1966) demonstrated that injection of pituitary extracts of Pacific salmon (Oncorhynchus kisutch and O. tshawytscha) into hypophysectomized Couesius plumbeus reversed the atrophy of interrenal tissue. Furthermore, a crude extract of pituitary glands from the Pacific salmon Oncorhynchus keta has been shown to deplete adrenal ascorbic acid in the rat, similar to mammalian ACTH (Rinfret and Hane, 1955).

Although it has been known for some time that the interrenal gland of teleosts is under pituitary control, the identification of the cell responsible for the secretion of a corticotrophic hormone has been a matter of speculation until very recently. A chromophobic, elongate cell, termed the epsilon cell (Olivereau and Ball, 1964), which is specifically

stained with MacConaill's (1947) lead-hematoxylin (Olivereau, 1964) has been suggested as the source of corticotrophic hormone (Olivereau and Ball, 1963, 1964; Ball and Olivereau, 1966; Ball and Baker, 1969). A palisade-like layer of lead-hematoxylin-positive cells in the rostral pars distalis bordering the neurohypophysis has been described in the adult sockeye salmon (van Overbeeke & McBride,1967) and rainbow trout (Fagerlund et al.,1968). These cells in salmonid pituitaries have been tentatively designated epsilon cells or corticotrops.

In order to ascertain the activity of teleostean interrenal tissue, many investigators have relied on subjective histological observations of interrenal response. Measurement of plasma cortisol levels has been employed as a method for quantitatively assessing pituitary-interrenal activity in adult salmonids (Fagerlund, 1967; Donaldson and McBride, 1967; Fagerlund et al., 1968). However, preliminary studies indicated that this method was impractical for determining the activity of the pituitary-interrenal axis of juvenile coho salmon in the present investigation, owing to the large number of coho fry required for a single determination. Numerous studies have shown that variations in the pituitary stimulation of the adrenal cortex of mammals are reflected in the width of the different cortical zones, and in the size of cell nuclei and nucleoli (Knobil et al., 1954; Miller, 1954; Holley, 1965; Molne, 1969). In teleosts, several investigators have utilized measurements of the size of interrenal nuclei to quantitatively assess interrenal activity (Krauter, 1958; van Overbeeke, 1960; Basu et al., 1965; Hanke and Chester Jones, 1966; van Overbeeke and Ahsan, 1966; Donaldson and McBride, 1967; Fagerlund and McBride, 1969). In the present investigations, measurements of interrenal nuclear diameters and estimates of interrenal cell sizes are utilized as indices of

interrenal gland activity in juvenile coho salmon. It was also decided
to ascertain whether nuclear diameters of epsilon cells in the juvenile coho
salmon would reflect changes in cellular activity.

Before investigating the activity of the pituitary-interrenal axis
of juvenile coho salmon with related changes in peripheral blood cell
counts in response to environment alterations, it was felt necessary to
first determine if this axis is capable of activity in young coho fry.
Therefore, in this investigation the histometric responses of the interrenal
cells and epsilon cells to injections of ACTH, cortisol and dexamethasone
have been determined, and correlated with alterations in numbers of
peripheral blood cell types. In an attempt to determine maximal responses
of these variables, dosages administered were pharmacological rather than
physiological.

MATERIAL AND METHODS

Experimental Animals

Seventy-five coho fry were captured from Bertrand Creek (Langley, B.C.) on June 22, 1968 by pole seine, and taken in buckets to the laboratory, where they were placed in holding tanks A, B and C. Twelve fish were put in each of A, B and C, remaining fry being placed in a large fibre-glass stock tank. All fry were fed frozen brineshrimp (Artemia sp.) daily throughout the experiments until sacrificed. Following a six week period of acclimation, all fry in holding tank A (stock sample) were sacrificed. Subsequently, twelve fry from holding tank B were transferred to experimental compartments, one fish per compartment. Each fish was lightly anaesthetized in 0.01% MS 222 (tricaine methane sulfonate; Kent Chemicals, Vancouver, B.C.) and weighed prior to transfer to individual compartments. Empty holding tanks were re-stocked (twelve fish per tank) with fry from the fibre-glass stock tank. All fish were held for at least four weeks in holding tanks before weighing and transfer to experimental compartments. Photocells were used to provide fry in all tanks and compartments with a natural photoperiod.

Description of Holding Tanks and Experimental Compartments

(a) Holding Tanks

The semi-circular fibre-glass stock tank, 111 cm. x 51 cm., had a water depth of 35 cm. This 200 liter tank was illuminated with fluorescent lighting providing an intensity of 1200 lux at the water surface. Light

Figure 1:   Holding-tank, equipped with a gravel bottom and assorted pieces of Plexiglass sheeting providing a means of artificial cover.

Figures 2 & 3: Experimental tanks, each of which is partitioned into three separate compartments with individual lighting, aeration and water supply.

Figure 4:   Experimental compartment, showing gravel bottom and artificial cover.

Figure 5:   Experimental compartment, as described in Section III. Note the size of the fish, relative to the size of the compartment.
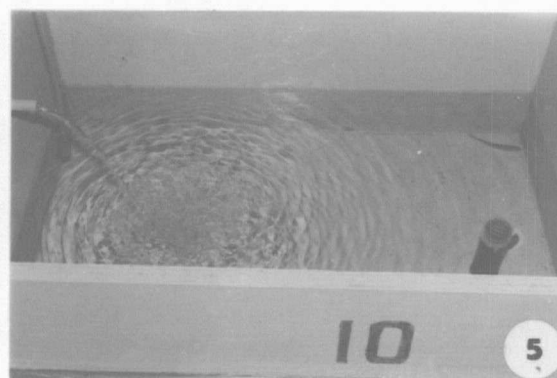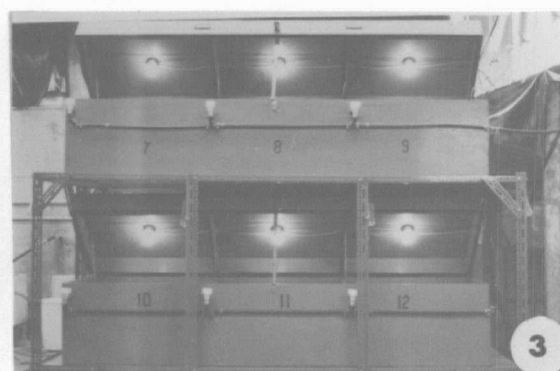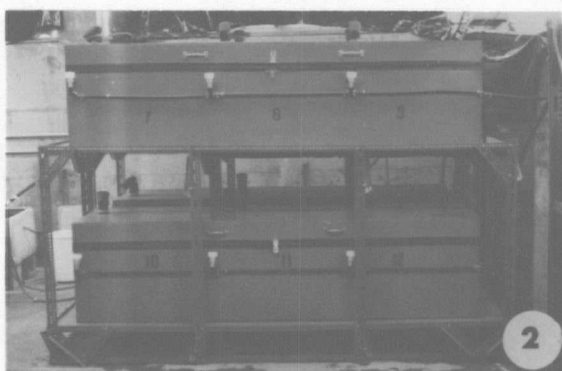
intensities were measured with a photovolt model 514M photometer. Holding tanks A, B and C were constructed of stainless steel, 105 cm. x 35 cm. x 30 cm. high, with a water depth of 23 cm. (Fig. 1). Each tank was painted with gray Rustoleum. These 85 liter tanks were illuminated with overhead fluorescent lights, providing 1100 lux at the water surface. Both the fibre-glass tanks and the holding tanks were provided with gravel bottoms. The stones ranged from 3 cm. to 7 cm. in diameter. Assorted sizes of Plexiglass sheeting were placed in all tanks, offering a means of artificial cover to the fry. The holding tanks were covered with sheets of fine-mesh wire, to prevent fry from escaping. The fibre-glass tank was covered with sheets of transparent plastic.

Water supply for all tanks consisted of dechlorinated water, continuously aerated. The fibre-glass tank received a flow rate of approximately 2 liters of water per minute, while the holding tanks A, B and C received 1 liter per minute.

(b) Experimental Compartments

Twelve large (80 cm. x 60 cm. x 45 cm.) light-tight compartments were constructed to contain individual fish. Four tanks, built with 3/4" plywood, were each partitioned into three units, and painted with non-toxic gray Rustoleum (Figs. 2 and 3). Each compartment received a separate dechlorinated water supply, maintained at a flow rate of 2 liters of water per minute throughout all experiments, and aerated within. Water was held at a depth of 15 cm., providing a volume of 72 liters of water per compartment.

Individual compartments were provided with a gravel bottom, and artificial cover in the form of two 12 x 10 cm. sheets of black Plexiglass

fused together at right angles (Fig. 4). A 40 W incandescent bulb mounted in the lid of each compartment provided 165 lux of illumination at the water surface. These lights were regulated by a time box connected to a dimmer switch, which caused the lights to brighten or dim gradually over a thirty minute period.

Experiments

Following transfer to individual experimental compartments from a holding tank, all fry were held for seven days prior to the initial injection. The fish were netted rapidly and lightly anaesthetized in 0.01% MS 222 before injection. Injections were made intraperitoneally just anterior to the cloaca with a 0.25 ml. syringe and No. 27-gauge needle (van Overbeeke & Ahsan, 1966). Several seconds were allowed before withdrawing the needle from the body. This precaution, combined with the light anaesthesia, ensured that little or no hormone was lost from the fish. In each experiment, fry were injected daily for seven consecutive days, followed by autopsy on the eighth day.

Injections of varying dosages of ACTH (mammalian corticotropin), cortisol acetate (FA) and Dexamethasone were administered in separate experiments to coho fry (see Table I). Since experiment 1 involved twenty-four fry, and experiment 2 twenty-two fry, more fish were utilized in each of these experiments than could be accommodated in the twelve compartments at one time. Therefore, experiments 1 and 2 were each performed in two identical parts consecutively, and the results from the same treatment pooled.

Autopsy Procedure

To terminate an experiment, a fish was removed rapidly from its

TABLE I

No. of exper- iment	No. of fish	Hormone injected	Duration, days	Daily dosage per gm. wt.		Total dosage received per gm. wt.		Daily volume received per gm. wt.
1	4	uninjected control	--	--		--		--
	4	*saline	7	--		--		0.01 ml.
	4	**ACTH	7	0.05	IU	0.35	IU	0.01 ml.
	4	ACTH	7	0.1	IU	0.7	IU	0.01 ml.
	4	ACTH	7	0.2	IU	1.4	IU	0.01 ml.
	4	ACTH	7	0.4	IU	2.8	IU	0.01 ml.
2	4	uninjected control	--	--		--		--
	4	saline	7	--		--		0.01 ml.
	5	'FA	7	2	ugm.	0.014	mg.	0.01 ml.
	4	FA	7	20	ugm.	0.14	mg.	0.01 ml.
	5	FA	7	200	ugm.	1.4	mg.	0.01 ml.
3	4	saline	7	--		--		0.01 ml.
	4	"Dexa.	7	1	ugm	0.007	mg.	0.01 ml.
	4	Dexa.	7	10	ugm.	0.07	mg.	0.01 ml.

*   Cortland's Fresh Water Fish Saline (Prosser & Brown, 1965)

**   Porcine ACTH (Nutritional Biochemical Co. lots No.4666 and No.7599) diluted with fish saline

'   Cortisol Acetate (Calbiochem. lot No.3871) suspended in fish saline

"   Dexamethasone Sodium Phosphate (Merck Sharp & Dohme lot No.3211M) dissolved in fish saline

compartment by netting, and "damp-dried". Wet weight was measured to the nearest tenth of a gram, and fork length was measured to the nearest millimeter. The caudal peduncle was wiped clean with 95% alcohol, dried and severed with surgical scissors. A blood smear was made, using glass slides pre-cleaned in 95% alcohol. The remaining blood from the caudal vessels was collected into a heparinized microhematocrit tube. Subsequently, the peritoneal cavity was opened and sex recorded. To avoid tissue damage, the head kidney containing the interrenal tissue was removed with the surrounding muscle mass, and fixed in Bouin's solution. Following this, the fish was decapitated; lower jaw, gill tissue, opercles and eyes were excised. To permit rapid fixation of the brain and pituitary gland, the dorsal surface of the skull was removed. The remaining brain and surrounding structures, with the pituitary gland in situ, was fixed in Bouin-Hollande-sublimate. The entire autopsy procedure was performed in less than five minutes. The total handling time from netting of the fish to the completion of the blood collection was usually less than one minute. Water temperature was recorded at the time of dissection.

The blood samples in the heparinized tubes were centrifuged at 12,500 rev./min. for 5 minutes in an Adams Microhematocrit Centrifuge (Clay-Adams CT-2900), and hematocrit was determined.

Histological Techniques

(a) Blood Smears

In preliminary studies, several blood-staining techniques were attempted, utilizing Jenner's, Giemsa's, Leishman's and Wright's stain. A procedure involving a combination of Giemsa's and Leishman's stain (G.W. Klontz, personal communication) was found to provide the most satisfactory

cellular discrimination and was subsequently used consistently. Details of this staining procedure are provided in Table I Appendix I.

The cells in each blood smear were counted under oil immersion in two separate manners. The first approach involved a routine differential white blood cell count. One hundred leucocytes were counted, and the percentage of each cell type recorded. The cells counted included small and large lymphocytes; juvenile, band and segmented neutrophils. Neutrophils were counted as a single class. The second approach entailed a more absolute measure of the number of individual leucocyte cell types in the total blood cell count. Utilizing an ocular grid, one thousand blood cells of all types (excluding thrombocytes) were counted. The cells counted included mature and immature red blood cells, large and small lymphocytes, juvenile, band and segmented heterophils. Since thrombocytes are sometimes in the blebbed state, or clumped, it was decided to count the number of thrombocytes observed within the area of the 1000 cells counted, rather than allow the variability of thrombocyte counts to invalidate the counts of other cell types.

Blood counts were confined to a rectangular area approximately 30 mm. by 10 mm. within the center of each smear in an attempt to reduce the source of error due to a possible preferential adhesion of a specific cell type to a particular region of the glass surface. An eight-key multiple counter was used for all counts.

(b) Head Kidneys and Pituitaries

Following fixation in Bouin's fluid for one week, head kidneys were dissected from surrounding tissues, dehydrated in ethanol and cleared in benzene in the usual manner and embedded in wax (Paraplast). Serial

transverse sections were cut at 5 μ. Alternate slides were than post-
chromed in 3% potassium dichromate overnight, followed by staining with
Mallory Heidenhain's Azan (Humason, 1962). Remaining slides were used for
RNA staining.

Tissue containing the pituitary gland was fixed in Bouin-Hollande-
sublimate for one week. After the usual procedures of dehydration and
clearing, the glands were vacuum-embedded in Paraplast. Serial 5 μ sections
were cut sagitally through the pituitary. Sections were stained with Lead
Hematoxylin (MacConaill, 1947).

(c) Staining of Interrenal Tissue for RNA

Transverse 5 u sections of head kidneys from coho fry injected
with either saline, ACTH or cortisol were stained with methylene blue for
RNA according to the method of Deitch (1964). Control sections from each
head kidney were treated with 1 N. HCl at 60°C. for 10 min. to extract
tissue RNA, while non-extracted sections were placed in distilled water
at 60°C. Subsequently, all sections were acetylated in 100% acetic
anhydride at 60°C. for 18 hours, followed by staining with methylene blue
chloride (Deitch, 1964). All sections for the various treatments were
stained at the same time.

Karyometry

(a) Interrenal Gland

From each head kidney, four sections were selected for measurement
providing an anterior to posterior cross-section of the distribution of
interrenal cells. Under low power magnification, a cluster of interrenal
cells was chosen indiscriminately from each section for measurement of 25
nuclei. Diameters of the interrenal cell nuclei were measured directly

with an ocular micrometer at 1000X magnification (oil immersion). By changing the focus of the microscope, a series of cross-sections through the preparation are seen. The area of the largest optical cross-section of each cell nucleus was measured. Since coho interrenal nuclei are elliptical, mutually perpendicular diameters (longest and shortest) of each nucleus were measured. A total of 100 interrenal nuclei from the head kidney of each fish were measured.

To determine if changes in interrenal nuclear diameter were correlated with changes in cell size, the number of interrenal nuclei within an area of interrenal tissue were counted. The area was defined arbitrarily as 16 squares of an ocular grid. At 1000X magnification, this encompasses an area of interrenal tissue of 450 μ^2. The number of interrenal nuclei within 10 such areas were counted for each head kidney under oil immersion, and the mean and standard error determined.

(b) Pituitary Glands

The diameters of 100 epsilon cell nuclei in the rostral pars distalis of the pituitary gland of each fish were measured directly at 1000X magnification with an ocular micrometer. Mutually perpendicular diameters of each nucleus were measured.

Statistical Evaluation of Results

Means and standard errors of nuclear diameters of interrenal and epsilon cells for each treatment were determined; a computer program provided a weighted mean, unbiased and of minimum variance (Brownlee, 1965).

In each experiment, Scheffe's test for multiple comparisons with unequal sample size (Brownlee, 1965) was used to compare mean values of interrenal cell nuclear diameters, numbers of interrenal nuclei per

defined area of interrenal tissue, and epsilon cell nuclear diameters from each treatment. Since Scheffe's test requires that variances be homogeneous, homogeneity of variances of means was established utilizing Bartlett's test (Brownlee, 1965). Means were considered to be significantly different if $p \leq 0.05$, and to be very significantly different if $p \leq 0.01$.

Variances of means of blood cell counts for different treatments were often not homeogeneous according to Bartlett's test. Therefore, Scheffe's test for multiple comparisons of means from each treatment could not be applied. Instead, mean blood cell counts for identical cell types from differing treatments within an experiment were compared by employing Student's t test. A computer program was written which initially utilized the F test to determine whether or not the variances of the two means were equal. If variances were considered to be the same, the two-sample t test was employed (Brownlee, 1965). If variances were considered to be unequal, Welch's approximation to the effective number of degrees of freedom (Brownlee, 1965) was used. Means were considered to be significantly different if $p \leq 0.05$, and to be very significantly different if $p \leq 0.01$.

Correlation coefficients relating all measured variables were determined for each experiment. The standard IBM Statistical system (1130-CA-06X) computer program which provides a correlation matrix of variables as well as summary statistics was employed. Variables were considered to be significantly correlated at the 95% level, and very significantly correlated at the 99% level.

RESULTS

Structure of the Interrenal Gland of Control Fry

The interrenal gland of coho fry is a diffuse organ, consisting of
small clumps of cells distributed irregularly in the hematopoietic tissue
of the head kidney, either surrounding the venous sinuses and small branches
of the posterior cardinal vein, or as discrete islets (Fig. 6). Clusters
of interrenal cells are surrounded by an extremely thin layer of connective
tissue. Melanophores are scattered throughout the head kidney. Unlike
more highly evolved teleosts, no chromaffin cells are associated with the
interrenal tissue.

The nuclei of interrenal cells are round to elliptical in cross-
section (Fig. 7), and contain one or two nucleoli. Chromatin material is
finely particulate and evenly dispersed. The cytoplasm contains evenly
dispersed, very fine granules, which are sensitive to both acidophilic
and basophilic dyes. Cell boundaries are difficult to discern.

Interrenal cell nuclei appear to be similar in size from cluster
to cluster; however, a one-way analysis of variance indicated that a
significant difference in variance of interrenal nuclear diameters (I.N.D.'s)
between clusters existed in 50% of the non-injected and saline-injected
controls. No anterior-posterior gradient was found when mean I.N.D.'s of
clusters measured from various regions within the head kidney were compared.
No histological or cytological differences were observed between the non-
injected and saline-injected controls for the various treatments. Likewise,
no differences were observed between the interrenal glands of control fish
and the stock-tank sample. Mitotic figures within the interrenal tissue

Figure 6:   Head kidney of a coho fry treated with 0.01 ml. saline/
              gm.wt./day for 7 days. Clusters of interrenal cells (IC)
              are distributed irregularly throughout the hematopoietic
              tissue (HT). Azan. X250.

Figure 7:   Higher magnification of interrenal cells shown in Figure
              6. Cell nucleoli small or inconspicuous. Azan. X900.

Figure 8:   Head kidney of a coho fry treated with 0.4 I.U. ACTH/gm.
              wt./day for 7 days. Note increased vacuolation (VAC) of
              hematopoietic tissue. Interrenal cells (IC) often
              associated with branches of the posterior cardinal vein
              (V). Azan. X250.

Figure 9:   Higher magnification of interrenal cells shown in
              Figure 8. Cells hypertrophied; also note prominent
              nucleoli. Azan. X900.

Figure 10:   Head kidney of a coho fry treated with 2 ugm. cortisol
              acetate/gm.wt./day for 7 days. Note shrinkage of
              interrenal cells (IC) and appearance of intercellular
              spaces within the cluster. Azan. X250.

Figure 11:   Higher magnification of interrenal cells shown in Figure
              10. Note vacuolated cytoplasm and irregularly-shaped
              dense, closely-apposed nuclei. Azan. X900.
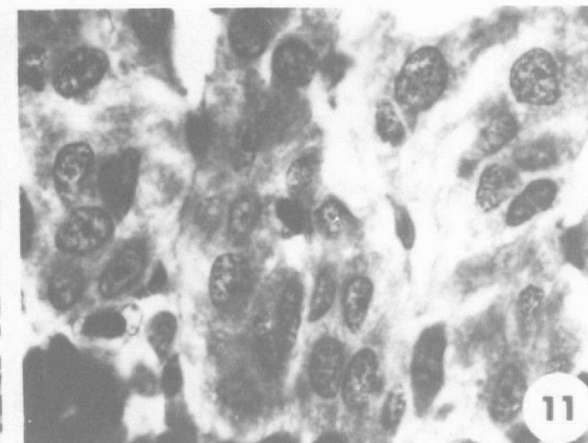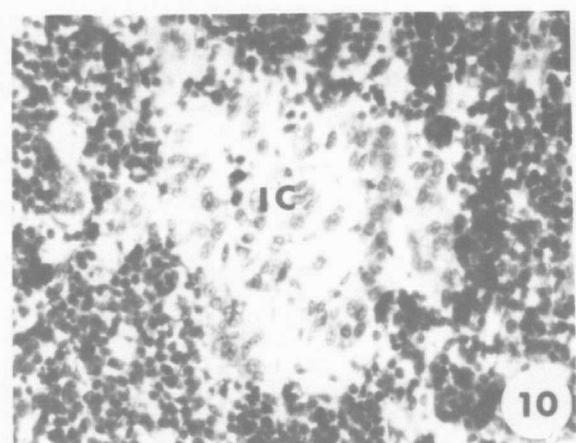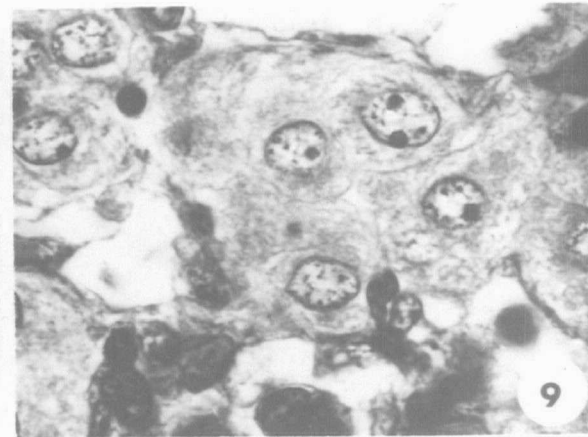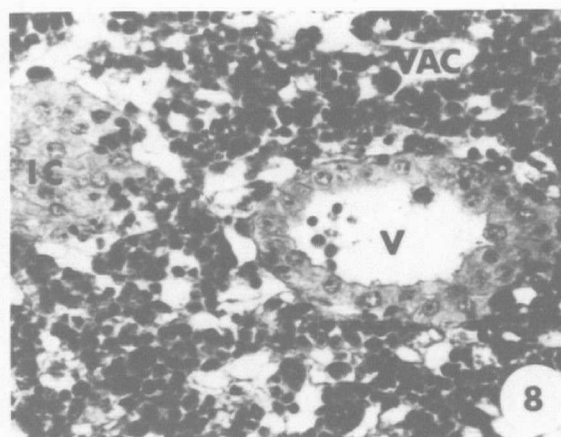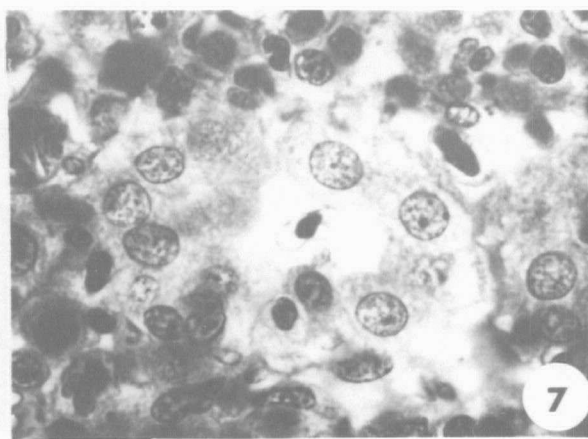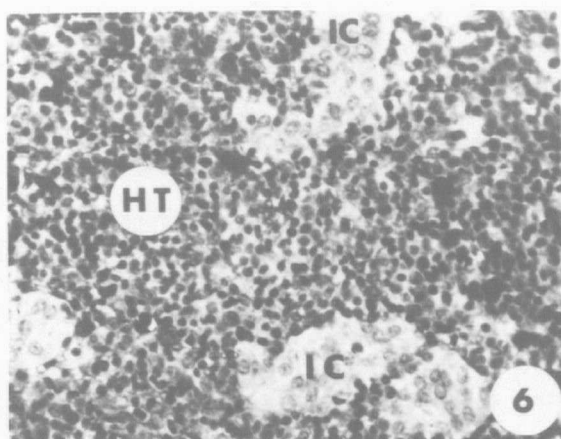
FIGURE 12:   A mid-sagittal section of the pituitary of a saline-

treated coho fry, showing the four defined regions:

Neurohypophysis (NH); pars intermedia (PI); proximal

pars distalis (PPD) and rostral pars distalis (RPD).

Note the follicles of eta cells (F) in the rostral pars

distalis, and the palisade layer of lead-hematoxylin-

positive epsilon cells (arrows) separating the

rostral pars distalis from the neurohypophysis.

SV: saccus vasculosus. Lead-hematoxylin. X275.
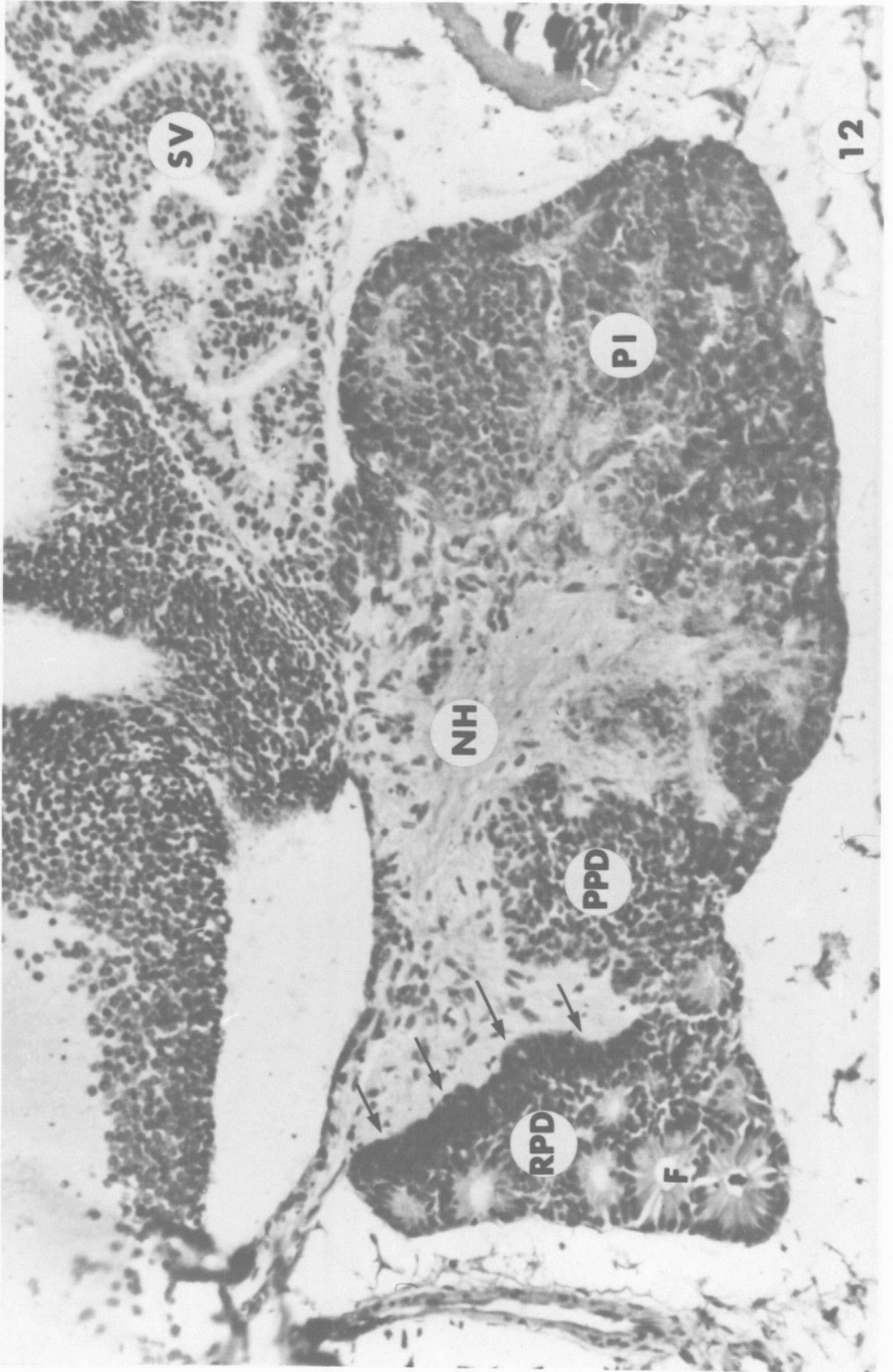
Figure 13:   A portion of the rostral pars distalis of the
             pituitary of a coho fry injected with 0.01 ml.
             saline/gm.wt./day for 7 days. Note the
             follicles (F) of lead-hematoxylin-negative "eta"
             cells and the palisade layer of strongly lead-
             hematoxylin-positive epsilon cells (arrows)
             bordering the neurohypophysis (NH). Lead-
             hematoxylin. X250.

Figure 14:   Higher magnification of the cells of the palisade
             layer shown in Figure 13. Note the intensity of
             cytoplasmic staining. Lead-hematoxylin. X900.

Figure 15:   A portion of the rostral region of the pituitary
             of a coho fry injected with 0.4 I.U. ACTH/gm.wt./
             day for 7 days. Note the lesser staining of
             the cytoplasm of the palisade-like layer of
             epsilon cells (arrows) compared with Figure 13.
             Lead-hematoxylin. X250.

Figure 16:   Higher magnification of the cells of the palisade
             layer shown in Figure 15. F: Follicle of eta cells.
             Lead-hematoxylin. X900.

Figure 17:   A portion of the rostral pars distalis of a coho fry
             injected with 1 ugm. dexamethasone/gm.wt./day for 7
             days. Note the lesser staining of the cytoplasm of
             the palisade layer of cells (arrows). Lead-
             hematoxylin. X250.

Figure 18:   Higher magnification of the lead-hematoxylin-
             positive cells shown in Figure 17. Lead-
             hematoxylin. X900.
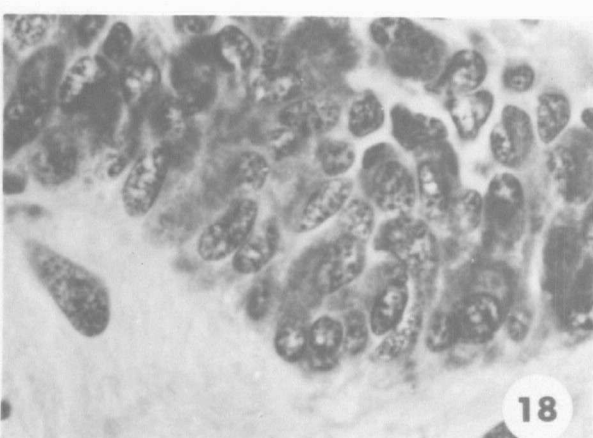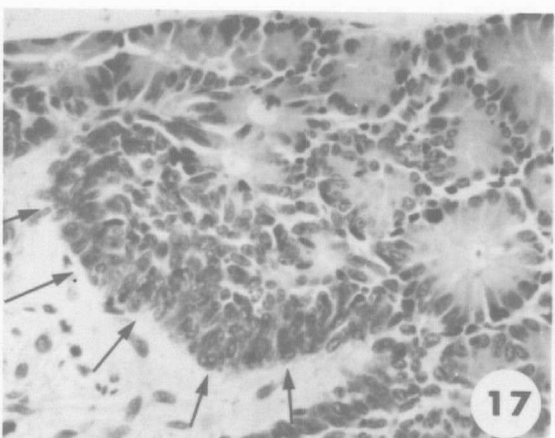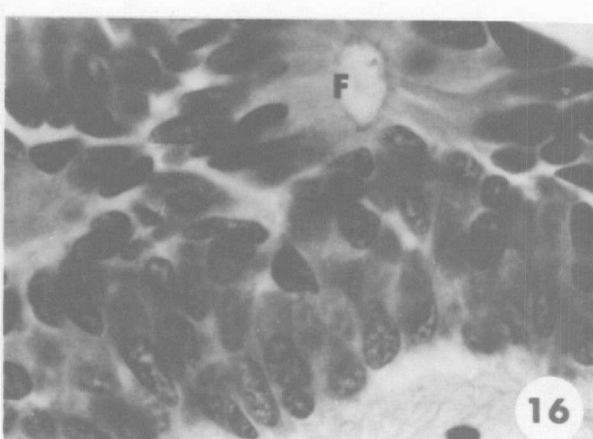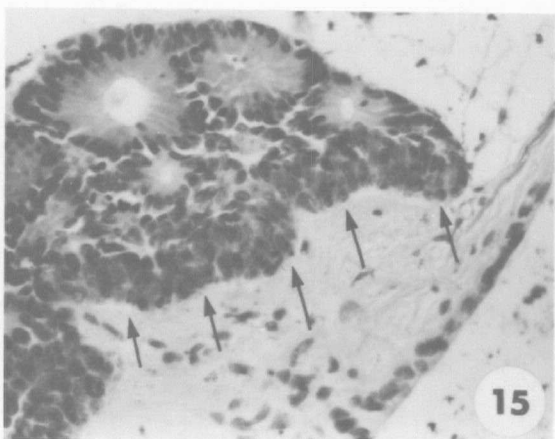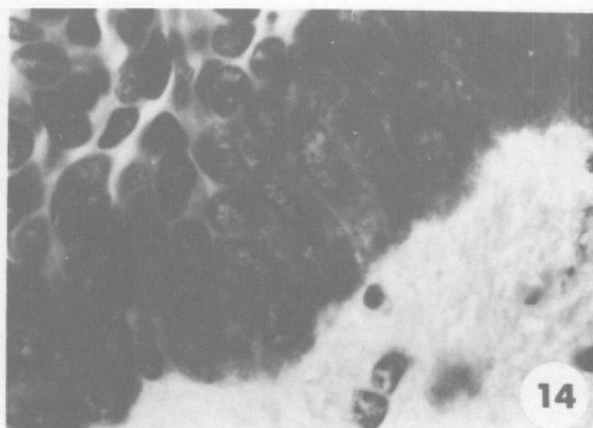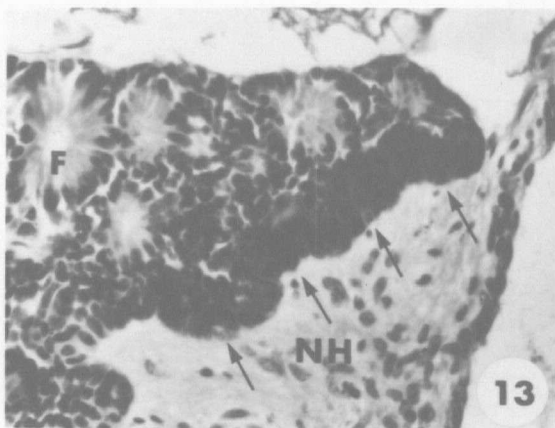
of control fish were extremely rare.

Structure of the Rostral Pars Distalis of Control Fry

The pituitary gland of coho fry has four defined regions: the rostral pars distalis (pro-adenohypophysis), proximal pars distalis (meso-adenohypophysis), pars intermedia (meta-adenohypophysis) and the neurohypophysis (Fig. 12). Mid-sagittal sections reveal a much greater anterior-posterior elongation of the gland when compared with similar sections of pituitaries from adult coho salmon (van Overbeeke, personal communication). The neurohypophysis, a large portion of the gland, consists mainly of fibres which interdigitate most extensively with the pars intermedia. Strands of cells in the pars intermedia, separated by ramifications of the neurohypophysis, contain two columnar cell types; one whose granular cytoplasm stains intensely with lead hematoxylin, the other whose cytoplasm remains completely chromophobic. These two cell types are randomly interspersed. The proximal pars distalis, a relatively small portion of the gland, consists of a mass of apparently undifferentiated cells, with small spherical nuclei, and very little chromophobe cytoplasm.

Most of the cells in the rostral pars distalis are columnar, and are arranged in follicles (Fig. 12 & 13). Follicular cell nuclei are elliptical, contain small, indiscriminate nucleoli, and are situated peripherally. Cytoplasm will not stain with lead hematoxylin. However, preliminary studies showed a strong affinity of the peripheral cytoplasmic granules for the acid fuchsin of Masson's trichrome stain or azocarmine (Mallory-Heidenhain's azan; Humason, 1962). A cell type with identical morphology and staining properties has been identified as the "eta" cell in the rostral pars distalis of the adult sockeye salmon pituitary gland (van Overbeeke and McBride, 1967).

Separating the follicular eta cells of the rostral pars distalis from the neurohypophysis is a palisade-like layer of cells, located at the dorsal postero-medial edge of the rostral zone (Figs. 12 & 13). This layer does not exceed three to four cells in thickness. Cell nuclei are elliptical with inconspicuous nucleoli, and are mostly arranged perpendicularly to the axis of the cell layer (Fig. 14). The cytoplasm is packed with very fine granules, which stain intensely blue-black with lead hematoxylin. In preliminary studies, the cytoplasm of these cells assumed an orange-brown color with Masson's technique, and appeared lavender with Mallory-Heidenhain's technique. Cells of a similar description, stainability and comparable location have been described previously in the pituitary gland of adult sockeye salmon (van Overbeeke & McBride, 1967; Fagerlund, McBride & Donaldson, 1968), and are referred to as "epsilon" cells.

A third type of cell is found in the rostral pars distalis in relatively small numbers, scattered amongst the eta cell follicles. This cell, possessing a small spherical nucleus and sparse chromophobe cytoplasm, appears identical to the cells of the proximal pars distalis.

No differences in cytology or affinity for stain were observed when the eta and epsilon cells of non-injected and saline-injected controls were compared.

Description of the Peripheral Blood Cell-types of Coho Fry

The peripheral circulation of fishes, unlike that of mammals, normally contains not only fully-differentiated blood cells, but also numerous immature cell-types in various stages of maturation. A typical blood smear of coho fry consists nearly exclusively of erythrocytes, although small and large lymphocytes, neutrophils and thrombocytes are also

Figure 19: Peripheral blood smear of a coho fry, showing mature erythrocytes (E) and two small lymphocytes (SL). Leishman-Giemsa. X1000.

Figure 20: Peripheral blood smear of a coho fry, showing three immature thrombocytes (IT). Note extremely dense nuclei, and traces of colorless cytoplasm. Leishman-Giemsa. X1000.

Figure 21: Peripheral blood smear of a coho fry, showing two small lymphocytes, and two mature thrombocytes (MT). A rim of colorless cytoplasm surrounds the elongate nuclei of the thrombocytes. Leishman-Giemsa. X1000.

Figure 22: Peripheral blood smear of a coho fry, showing a large lymphocyte (LL). Notice eccentric nucleus and extensive blue cytoplasm. Leishman-Geimsa. X1000.

Figure 23: Peripheral blood smear of a coho fry, showing a band neutrophil (BN). Note horseshoe-shaped nucleus and granulated cytoplasm. Leishman-Giemsa. X1000.

Figure 24: Peripheral blood smear of a coho fry, showing a mature segmented neutrophil (SN) and a mid-polychromatocyte (MP). The segmented neutrophil has a trilobed nucleus, and a finely-granular grey cytoplasm. The mid-polychromatocyte, with its blue-grey cytoplasm, has a distinct, radially-arranged chromatin pattern. Leishman-Giemsa. X1000.

Figure 25: Peripheral blood smear of a coho fry, showing a segmented neutrophil (SN) and two late-polychromatocytes (LP). Cytoplasm of the ovoid late polychromatocytes is grey. Leishman-Giemsa. X1000.

Figure 26: Peripheral blood smear of a coho fry suffering from tail rot, showing a macrophage (M). Note the pale purple nucleus with diffuse chromatin, and the vacuolated blue-grey cytoplasm containing ingested material. Leishman-Giemsa. X1000.
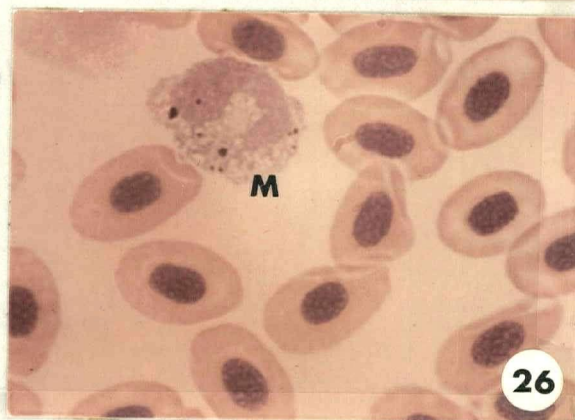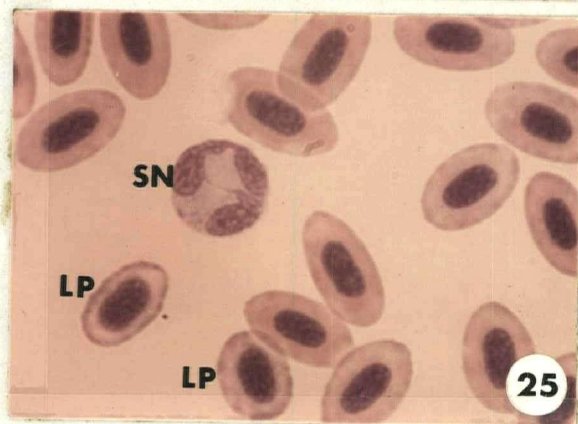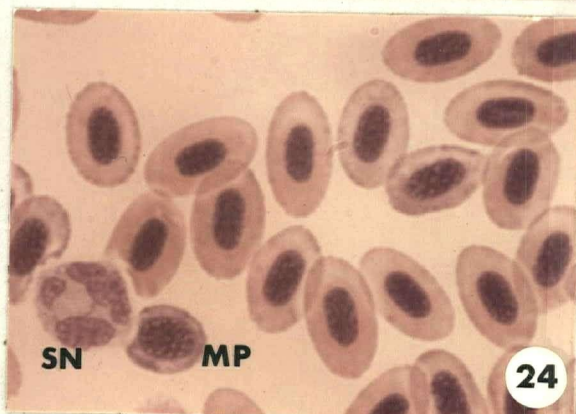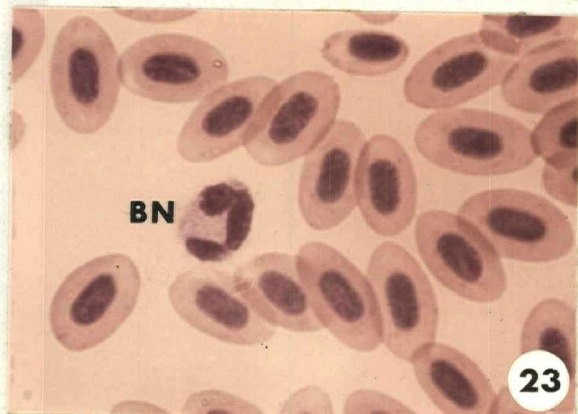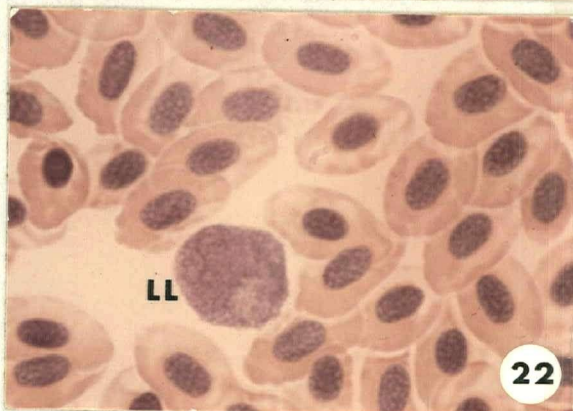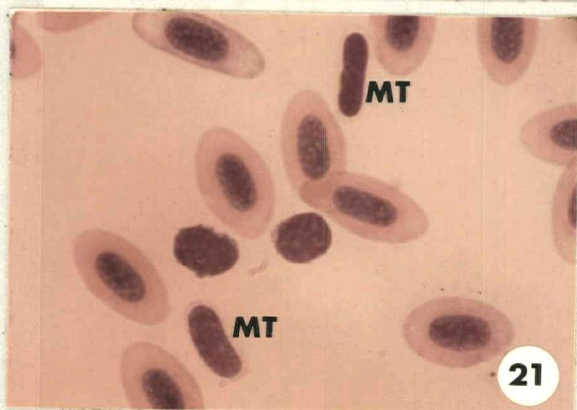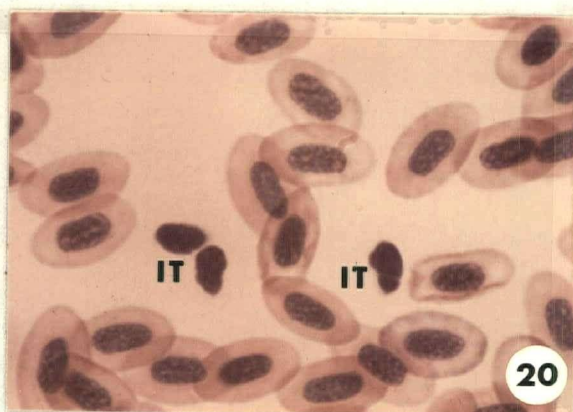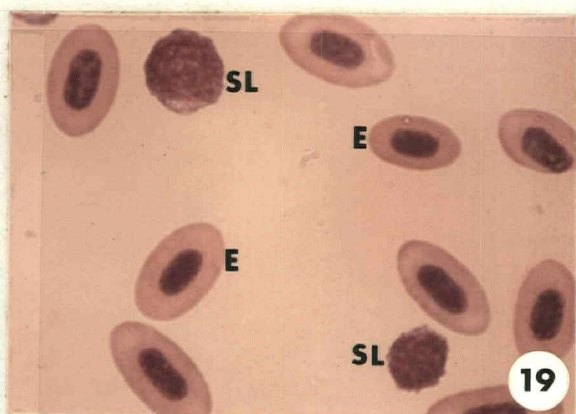
present. Since no suitable account of salmonid blood cells exists in the
literature, a description of the various blood cell types as they appear in
normal smears stained with Leishman-Giemsa follows.

The mature erythrocyte (E) is an elongated cell 12-16 μ long by 7-9
μ wide (Fig. 19). The nucleus, averaging 6.5 μ X 4 μ, stains purplish-blue.
Chromatin particles are small, and form a tightly-meshed network, surrounded
by a very thin nuclear membrane. Cytoplasm stains salmon-pink, and is
usually clear, although small dots or short rods are sometimes present
throughout. Cells undergoing amitotic divisions have been observed, although
very rarely. Also, erythroplastids (anucleated red blood cells) and
microcytes (small isolated nuclei) are found infrequently.

The late polychromatocyte (LP) appears normally in the circulation
in small numbers. This is an ovoid cell 10-15 μ long and 7-10 μ wide. The
ovoid nucleus appears similar to that of mature erythrocytes. Cytoplasm
stains a homogeneous grey (Fig. 25).

The mid-polychromatocyte (MP), found infrequently in normal smears,
is a spheroid cell 7-11 μ in diameter (Fig. 24). The round nucleus,
centrally located, has a distinct pattern of chromatin compacted into
thick, radially-arranged strands. The nuclear membrane is obvious.
Cytoplasm stains light blue-grey, and is more intense near the periphery.

The early polychromatocyte is a spheroid cell 7-11 μ in diameter,
with a round, centrally located nucleus surrounded by a fairly prominent
nuclear membrane. The cytoplasm stains a deeper blue-grey than that of the
mid-polychromatocyte. These cells are very rarely found in smears of
peripheral blood.

The small lymphocyte (SL) is the predominant leucocyte in the
peripheral blood of coho fry. This is a spheroid cell 6.5-10 μ in

diameter (Fig. 19). The reddish-purple round nucleus, occupying most of the cell, contains a distinct chromatin pattern of alternating dark and light areas. A narrow rim of blue cytoplasm is present, which often contains azurophilic granules. Pseudopodia are sometimes evident. Occasionally, small lymphocytes with indented nuclei and a small area of pink cytoplasm within the cleft are found.

The large lymphocyte (LL) is a more ovoid cell, 10-15 µ in diameter (Fig. 22). The reddish-purple nucleus is eccentric, and often irregular or kidney-bean shaped. Chromatin material consists of coarsely-meshed strands. Intra-nuclear vacuoles are sometimes observed. The blue cytoplasm is more extensive than that of small lymphocytes, and often contains vacuoles.

The neutrophil is the representative cell of the granulocytic series. Mature or segmented neutrophils (SN) are spheroid cells 10-15 µ in diameter (Figs. 24 & 25). The reddish-purple nucleus is lobulated, with each lobule connected by a thin filament of nuclear material. Chromatin consists of irregular patches of light and dark-staining material. Nuclei are found with from two to six lobulations. Cytoplasm is grey and very finely granular.

Juvenile and band neutrophils are the immature forms of neutrophils found in the circulation. The juvenile neutrophil is a spheroid to ovoid cell, measuring 10-14 µ in diameter. The nucleus is markedly indented and the chromatin material is quite compact. Cytoplasm is grey and finely granular. The band neutrophil (BN) is a spheroid cell 10-14 µ in diameter (Fig. 23). The nucleus is more elongated than that of the juvenile neutrophil, and is often horseshoe or S-shaped. Chromatin patterns resemble those of segmented neutrophils. The grey cytoplasm is finely granular.

Thrombocytes are found in several stages of maturity, ranging from immature spherical or slightly oval forms to elongated mature forms. The immature thrombocyte (IT), measuring 5.5 x 4.5 μ, contains an extremely dense, reddish-purple nucleus, exhibiting very little chromatin pattern (Fig. 20). A small amount of colorless or pale blue cytoplasm surrounds the nucleus, or forms a pseudopod at the pole. In slightly more mature thrombocytes, measuring 7.5 x 3.5 μ, a sharp indentation in the nucleus appears mid-way between the poles. Mature, elongated thrombocytes (MT), measuring 10.5 x 3.5 μ, contain a rod-shaped nucleus, rounded at both ends which is surrounded by a rim of colorless cytoplasm (Fig. 21). This nucleus often has a smooth indentation mid-way between the poles. The density of chromatin appears lighter with cell maturity, giving it an uneven appearance. A few purple granules are scattered randomly in the cytoplasm of thrombocytes of all stages. Intermediate and mature thrombocytes are sometimes found in clumps with their clear cytoplasm fused.

No basophils, eosinophils or monocytes are present in the circulation of coho fry. Also, no macrophages are normally found. However, numerous macrophages were present in the circulation of coho fry suffering from tail rot, an infection thought to be caused by bacteria (Davis, 1961).

The macrophage (M) is a speroid to ovoid cell, measuring 13-23 μ in diameter (Fig. 26). Its pale purple nucleus has a diffuse chromatin pattern. The nucleus is variable in shape, being eccentrically located within the cell. The extensive blue-grey cytoplasm contains several vacuoles, with frequent inclusions of ingested bacteria.

The Effect of ACTH Injections on the Pituitary-interrenal Axis

ACTH injections produce striking changes in the histology of the head kidney. A noticeable increase in vascularization occurs, with the appearance of many

blood sinuses. Also, spaces appear in the hematopoietic tissue (Fig. 8); these are apparently associated with the atrophy and disappearance of the lymphocytes. Even with injection of low dosages of ACTH (0.05 IU. ACTH./gm.wt.), an increased vacuolation of the hematopoietic tissue is observed; however, this effect is more pronounced with dosages of 0.2 and 0.4 IU. ACTH/gm.wt.

A marked hyperplasia of the interrenal tissue within the head kidney results from ACTH injections. Clusters of interrenal cells are larger than those of control fish. Much of the proliferated interrenal tissue becomes associated with small branches of the posterior cardinal vein and the blood sinuses. Many interrenal cells become follicularly arranged.

The tissue hyperplasia is accompanied by a striking hypertrophy of interrenal cells (Fig. 9). Interrenal nuclei appear markedly enlarged. Chromatin material is finely particulate, evenly dispersed, and stains less intensely than that of controls. Nucleoli are much more prominent than in controls. Cytoplasm appears unaltered, although more extensive than controls. Cell boundaries are readily discernible. Mitotic figures are infrequently observed in interrenal tissue of fry injected with ACTH, although much more numerous than in controls.

The hypertrophy of interrenal cells due to ACTH injections appeared to be uniform throughout the head kidney. No preferential response of cells within a particular cluster was observed. A one-way analysis of variance indicated that a significant difference in variance of interrenal nuclear diameters between clusters existed in only 35% of the head kidneys of fry injected with ACTH, compared with a significant difference in 50% of the control fish. Therefore, the response of inter-renal cells to exogenous ACTH is a uniform one.

The palisade-like layer of epsilon cells in the rostral pars distalis of ACTH-treated fish stains less intensely with lead hematoxylin when compared to controls (see Figures 13, 14, 15 & 16). This layer ramains 3-4 cells in thickness. Epsilon cell nuclei appear similar in size to controls, with inconspicuous nucleoli. Of the other cell types present in the pituitary, including those of the pars intermedia, none show any apparent change following ACTH treatment.

The mean diameters of the interrenal cell nuclei for all ACTH-treated and control coho fry are summarized in Figure 27 and Table II. Utilizing Scheffe's test for multiple comparisons, the mean interrenal nuclear diameters for the stock-tank sample, uninjected controls and saline-injected controls did not differ significantly. However, nuclear diameters were very significantly increased for all dosages of mammalian ACTH. Furthermore, each higher dosage of ACTH produced a very significant increase in mean diameter.

Interrenal cell size was estimated by determining the mean number of nuclei within a defined area of interrenal tissue. According to Scheffe's test, the mean number of interrenal cell nuclei for the stock-tank sample was very significantly larger than that for either the non-injected or saline-injected controls. Saline injections did not alter the mean number of interrenal nuclei (No. Nu.) significantly from that of uninjected controls (Fig. 30 and Table II). However, all dosages of ACTH caused a very significant decrease in mean No. Nu. compared with saline-injected controls, reflecting a significant increase in interrenal cell size. Increasing dosages consistently resulted in an increased cell size, although a difference in effect was only significant when mean No. Nu. of fish receiving 0.05 I.U. and 0.1 I.U. ACTH/gm.wt./day were compared.

TABLE II

EFFECT OF ACTH-INJECTIONS ON INTERRENAL NUCLEAR DIAMETERS (I.N.D.), NUMBER
OF INTERRENAL NUCLEI PER DEFINED AREA OF INTERRENAL TISSUE (NO.NU.),
AND EPSILON CELL NUCLEAR DIAMETERS (E.N.D.) OF COHO FRY

Number of Fish	Treatment/ gm.wt./day for 7 days	I.N.D. (microns) Mean ± S.E.	NO.NU. Mean ± S.E.	E.N.D. (microns) Mean ± S.E.
11	Stock sample	6.07 ± 0.01	28.6 ± 0.7	5.34 ± 0.03
4	Uninjected control	6.12 ± 0.02	21.4 ± 0.3	5.63 ± 0.04
4	Saline	6.06 ± 0.02	23.7 ± 0.8	5.56 ± 0.05
4	0.05 I.U. ACTH	6.52 ± 0.02	17.3 ± 0.5	5.12 ± 0.03
4	0.1 I.U. ACTH	6.69 ± 0.03	13.9 ± 0.2	5.23 ± 0.03
4	0.2 I.U. ACTH	7.04 ± 0.03	12.4 ± 0.6	5.37 ± 0.03
4	0.4 I.U. ACTH	7.43 ± 0.02	11.3 ± 0.6	5.29 ± 0.04

TABLE III

EFFECT OF CORTISOL ACETATE (FA) AND DEXAMETHASONE (DEX) INJECTIONS ON
INTERRENAL NUCLEAR DIAMETERS (I.N.D.), NUMBER OF INTERRENAL NUCLEI
PER DEFINED AREA OF INTERRENAL TISSUE (NO.NU.), AND EPSILON CELL
NUCLEAR DIAMETERS (E.N.D.) OF COHO FRY

Number of Fish	Treatment/ gm.wt./day for 7 days	I.N.D. (microns) Mean $\pm$ S.E.	NO.NU. Mean $\pm$ S.E.	E.N.D. (microns) Mean $\pm$ S.E.
4	Uninjected control	6.16 $\pm$ 0.02	21.5 $\pm$ 1.6	5.50 $\pm$ 0.03
4	Saline (FA)	6.24 $\pm$ 0.02	20.5 $\pm$ 1.0	5.41 $\pm$ 0.03
5	2 ugm. FA	6.06 $\pm$ 0.02	29.5 $\pm$ 0.4	5.12 $\pm$ 0.03
4	20 ugm. FA	5.98 $\pm$ 0.02	28.2 $\pm$ 0.7	5.35 $\pm$ 0.03
5	200 ugm. FA	6.10 $\pm$ 0.03	28.9 $\pm$ 0.7	5.12 $\pm$ 0.03
4	Saline (DEX)	6.28 $\pm$ 0.02	24.5 $\pm$ 0.7	5.33 $\pm$ 0.03
4	1 ugm. DEX	6.12 $\pm$ 0.02	26.8 $\pm$ 1.0	5.34 $\pm$ 0.03
4	10 ugm. DEX	6.14 $\pm$ 0.02	29.3 $\pm$ 1.0	5.30 $\pm$ 0.04

FIGURE 27:   Effect of ACTH on nuclear diameters of interrenal

and epsilon cells, and on small-lymphocyte, thrombo-

cyte and neutrophil counts.
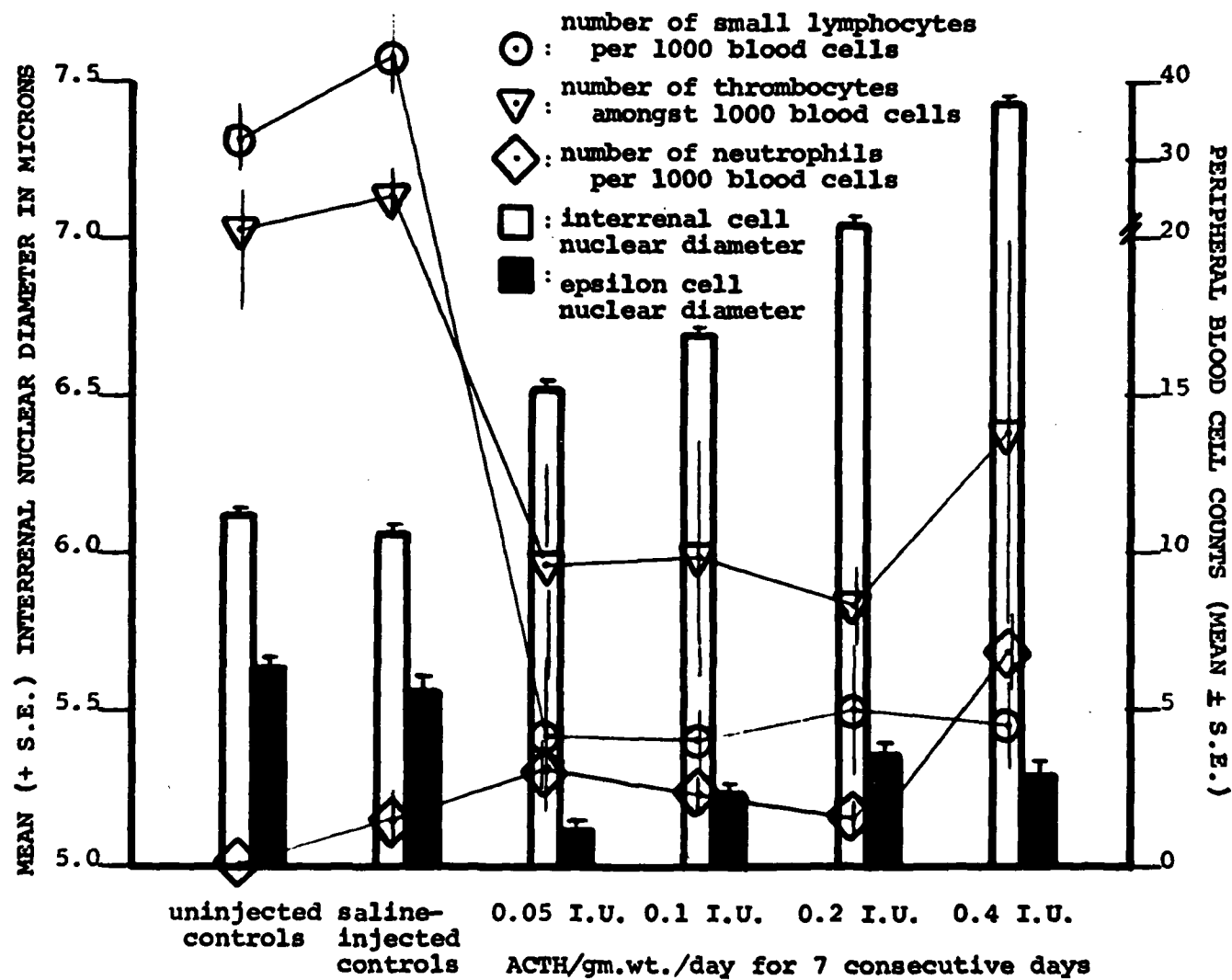
FIGURE 28:   Effect of cortisol acetate on nuclear diameters of
interrenal and epsilon cells, and on small-lymphocyte,
thrombocyte and neutrophil counts.
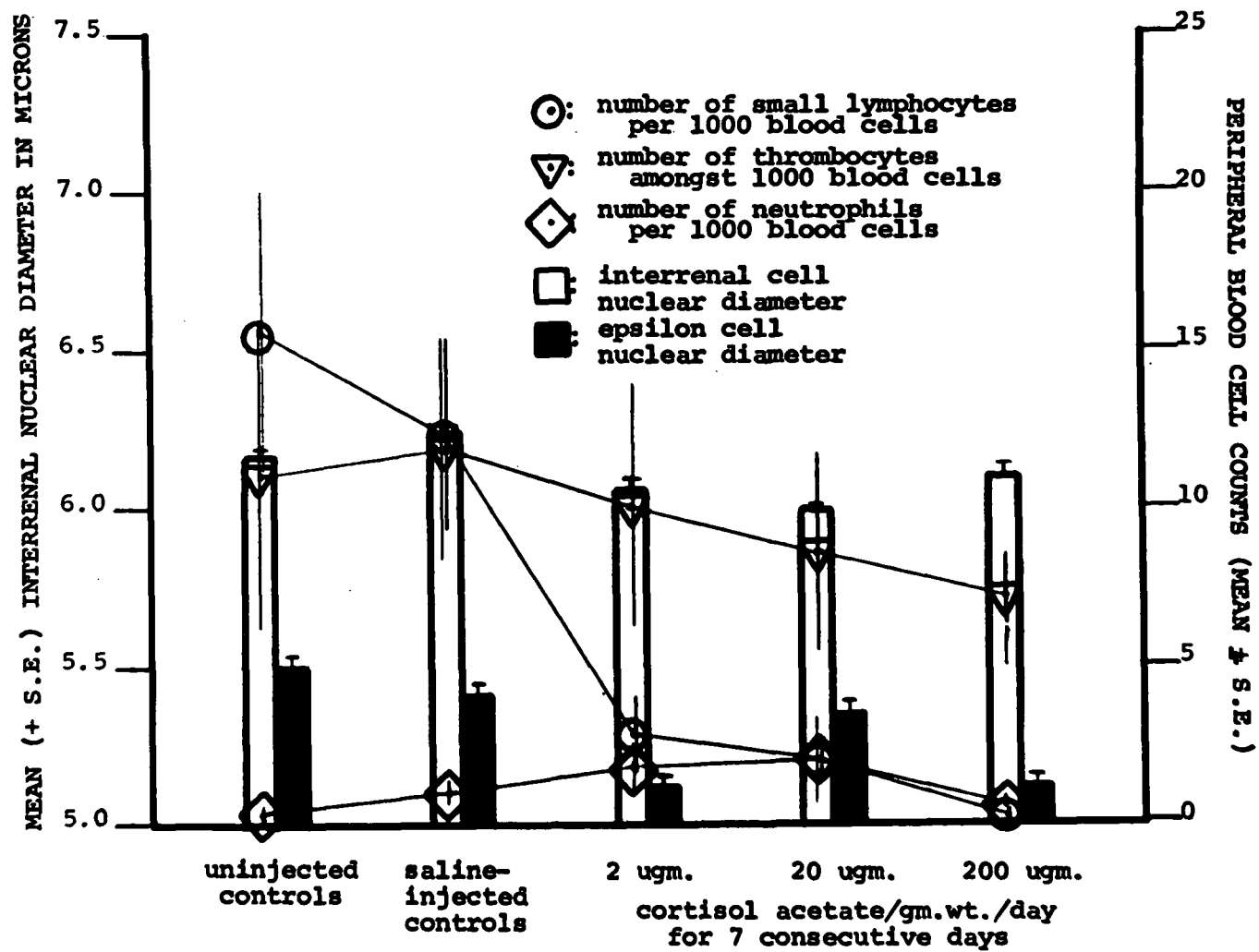
FIGURE 29: Effect of dexamethasone on nuclear diameters of interrenal and epsilon cells, and on small-lymphocyte, thrombocyte and neutrophil counts.
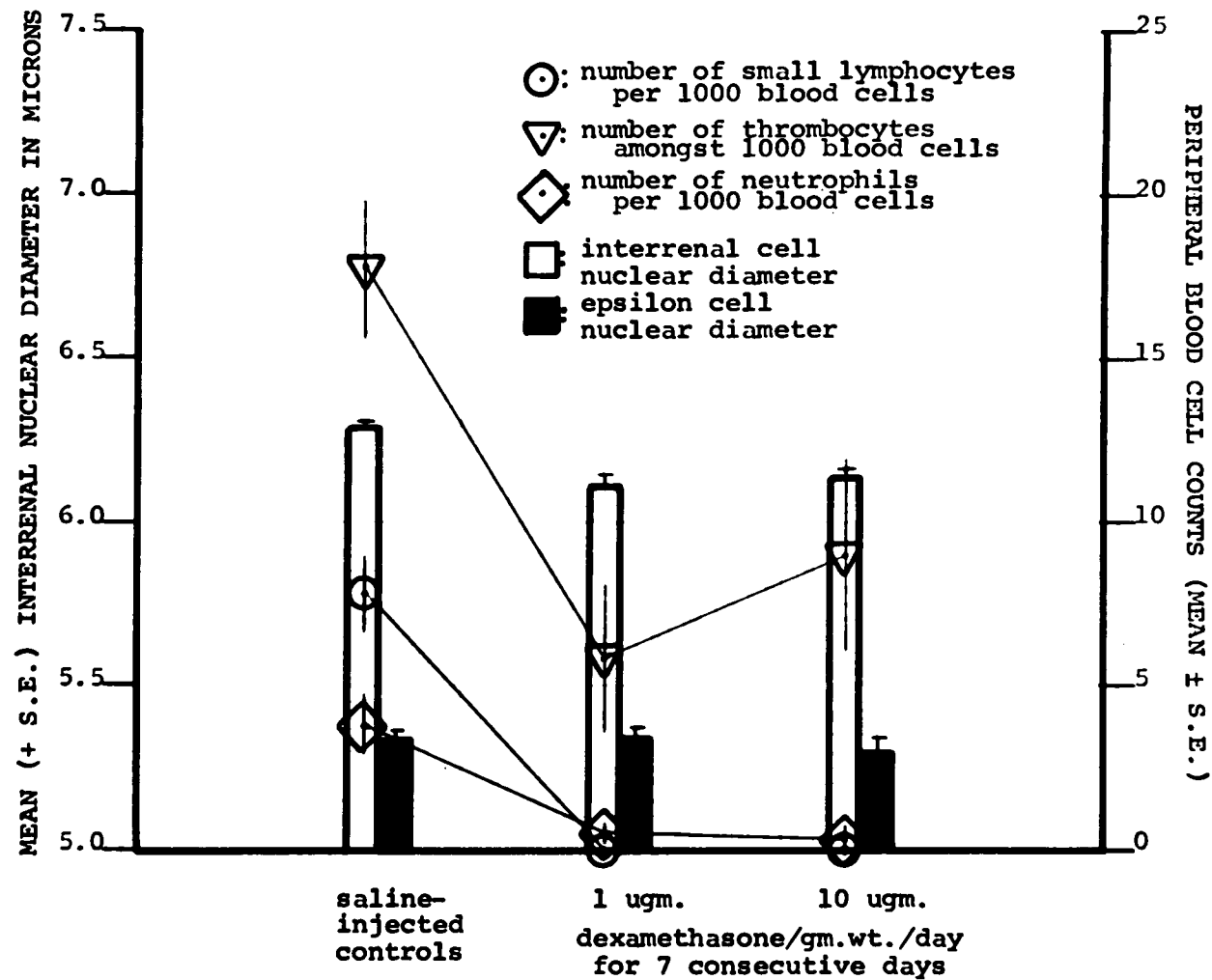
FIGURE 30:   Effect of ACTH on interrenal cell size*.
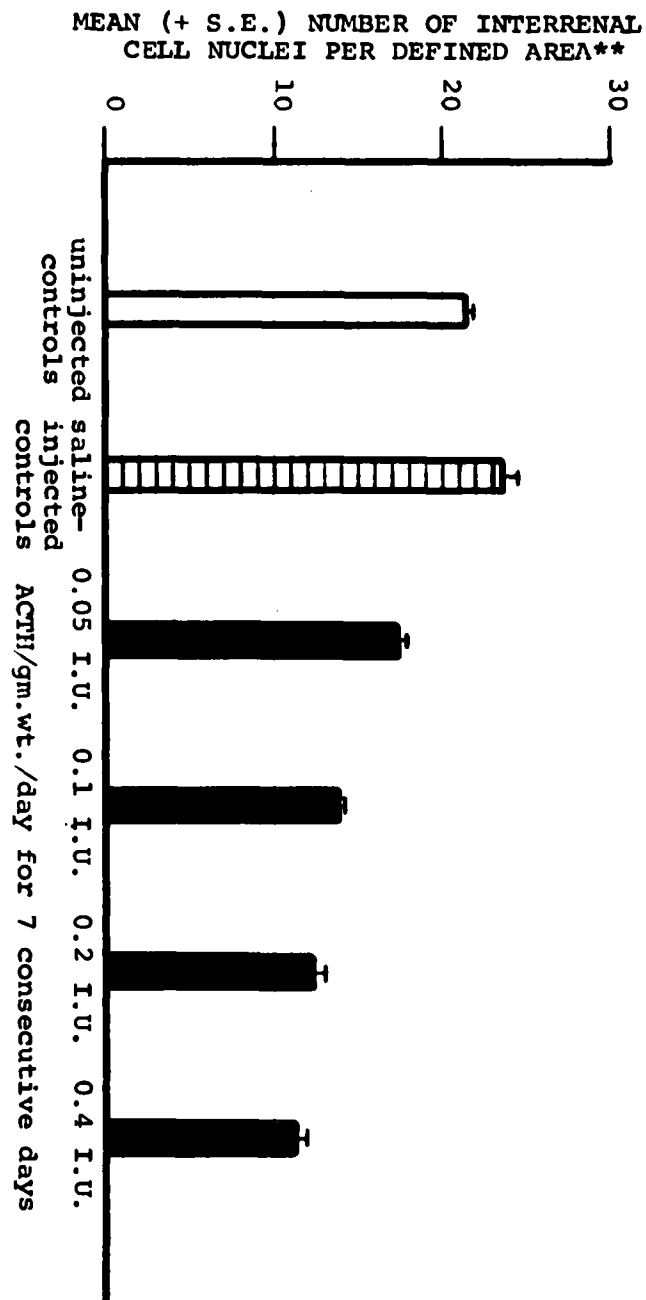
MEAN (+ S.E.) NUMBER OF INTERRENAL
CELL NUCLEI PER DEFINED AREA**

0   10   20   30

uninjected
controls

saline-
injected
controls

0.05 I.U.   0.1 I.U.   0.2 I.U.   0.4 I.U.

ACTH/gm.wt./day for 7 consecutive days

*Interrenal cell size is inversely related to the
number of interrenal cell nuclei per defined area.

**Defined area is 450 μ^2 of interrenal tissue.

FIGURE 31:   Effect of cortisol acetate (FA)
              on interrenal cell size*.

FIGURE 32:   Effect of dexamethasone
              (DEX) on interrenal cell
              size*.
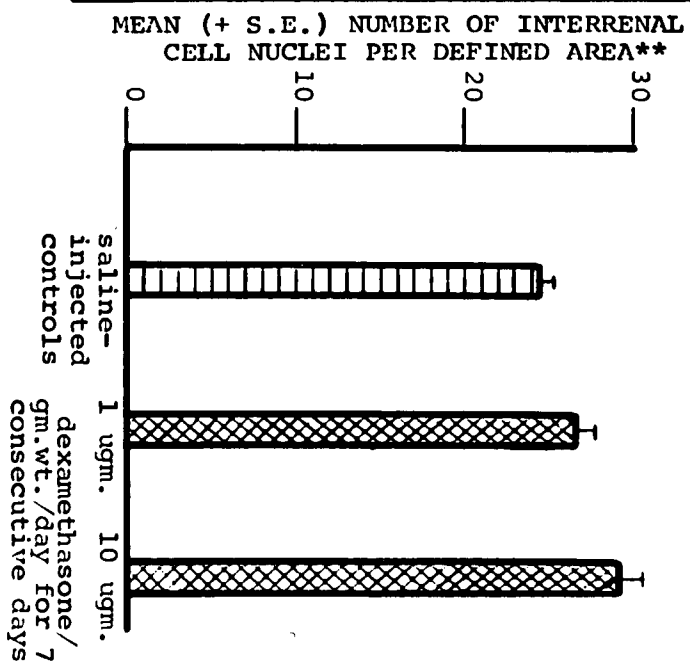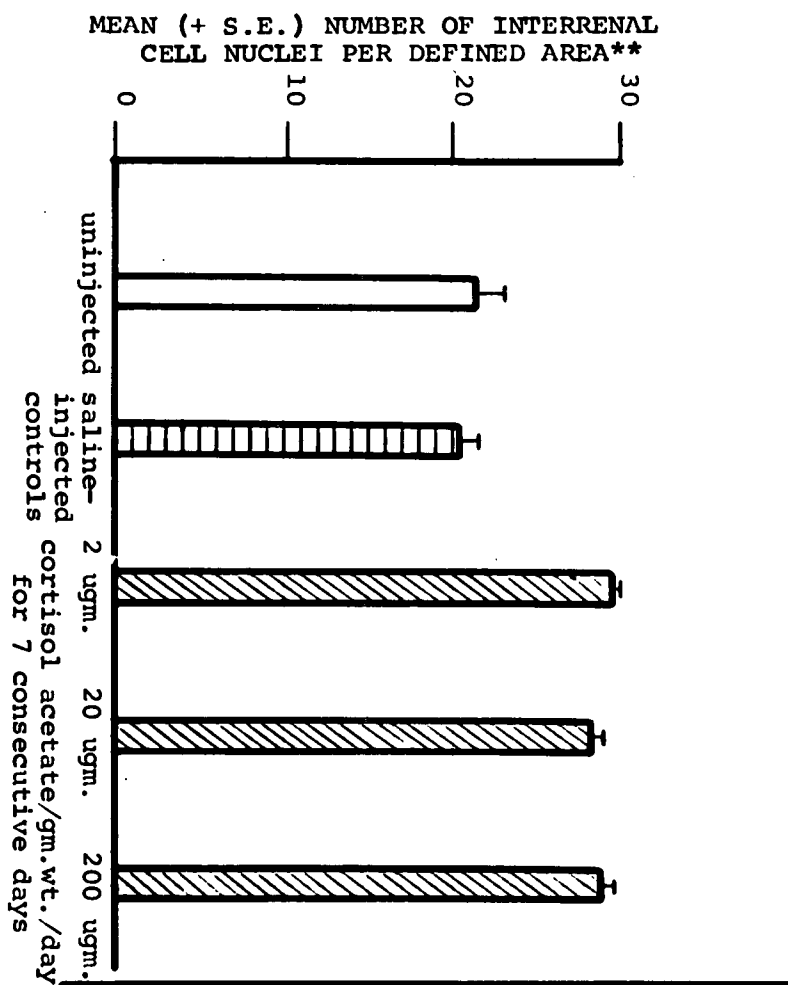
MEAN (+ S.E.) NUMBER OF INTERRENAL
CELL NUCLEI PER DEFINED AREA**

0   10   20   30

uninjected
controls

saline—
injected

2 ugm.

20 ugm.

200 ugm.

cortisol acetate/gm.wt./day
for 7 consecutive days

MEAN (+ S.E.) NUMBER OF INTERRENAL
CELL NUCLEI PER DEFINED AREA**

0   10   20   30

saline—
injected
controls

1 ugm.

10 ugm.

dexamethasone/
gm.wt./day for 7
consecutive days

*Interrenal cell size is inversely related to the
 number of interrenal cell nuclei per defined area.

**Defined area is 450 μ^2 of interrenal

Mean nuclear diameters of epsilon cells for control and ACTH-treated fish are presented in Figure 27 and Table II. The mean nuclear diameters for uninjected and saline-injected controls did not differ significantly. However, mean epsilon cell nuclear diameters were smaller than nuclear diameters of controls for all dosages of ACTH. This decreased nuclear size compared to saline-injected fish was significant according to Scheffe's test for fry receiving 0.05, 0.1 and 0.4 I.U. ACTH/gm.wt./day, but not for fry receiving 0.2 I.U. ACTH/gm.wt./day. However, this difference was significant according to Student's t test. The mean epsilon cell nuclear diameter of the stock-tank sample was very significantly smaller than that of the uninjected controls, and significantly smaller than that of the saline-injected controls.

For the various treatments, no consistent significant differences in mean interrenal nuclear diameters, numbers of interrenal nuclei per defined area of interrenal tissue or epsilon cell nuclear diameters could be attributed to the sex of the fish.

The Effects of Cortisol and Dexamethasone Injections on the Pituitary-Interrenal Axis

In comparing the histological structure of the interrenal tissue of the cortisol-injected fish with the controls (Figs. 10 & 11), it is apparent that the interrenal tissue of the former has undergone considerable atrophy. Shrinkage of the cells is obvious. Many small intercellular spaces appear within a cluster. Nuclei are smaller and are very irregular in shape. Chromatin stains more densely, and is somewhat clumped. Nuclei are packed closely together. Nucleoli are variable in size, but are usually small or indistinct. Cytoplasm stains irregularly,

and is often vacuolated. Cell boundaries are very difficult to discern. Clusters of interrenal cells are often invaded with blood cells. No histological differences due to variation in dosage of cortisol acetate were observed.

Dexamethasone treatment affects the histology of the interrenal tissue similarly. Shrinkage of the cells, appearance of intercellular spaces, nuclear distortion and shrinkage, vacuolation and variable staining of the cytoplasm occur consistently. Nucleoli are invariably smaller than controls. Interrenal tissue appeared similar for both dosages of dexamethasone. Mitotic figures were never observed in interrenal tissue of fish treated with either dexamethasone or cortisol.

The atrophy due to cortisol and dexamethasone of interrenal tissue appeared to be consistent throughout the head kidney, with no obvious differential response of cells from cluster to cluster. A one-way analysis of variance indicated that a significant difference in variance of inter-renal nuclear diameters between clusters was present in 50% of the treated fish, which is consistent with the normal variance of controls.

Hematopoietic tissue in the head kidney of fry treated with cortisol or dexamethasone is slightly more vacuolated than that of controls. Most lymphocytes within the hematopoietic tissue were atrophic. However, the vacuolation is far less extensive than that produced by ACTH injections. No increased vascularization of the head kidney due to cortisol or dexamethasone administration was evident.

Although the epsilon cells of all the controls stained moderately to intensely with lead haematoxylin, those of the cortisol and dexamethasone treated fish appeared only lightly granulated (Figs. 13 & 14, 17 & 18). The palisade layer of epsilon cells did not vary in thickness from controls.

Cytological differences in the epsilon cells other than that of granulation were not apparent. None of the other pituitary cell-types, including the lead hematoxylin-positive cells of the pars intermedia, showed any evidence of change following cortisol or dexamethasone injections.

Mean nuclear diameters of the interrenal cells of fry injected with cortisol and dexamethasone are presented in Figures 28 and 29, and Table III. Nuclear diameters of uninjected and saline-injected controls did not differ significantly. However, mean diameters were significantly decreased for all dosages of cortisol acetate compared to saline-injected controls. Likewise, injections of both 1 and 10 ugm. dexamethasone/gm.wt./day very significantly reduced mean interrenal nuclear diameters from that of saline-injected controls. Low dosages of either cortisol or dexamethasone were equally as effective in reducing mean diameters.

The effect of cortisol and dexamethasone injections on interrenal cell size is represented in Figures 31 and 32, and Table III. All dosages of cortisol acetate produced a very significant increase in mean number of interrenal cell nuclei per defined area of interrenal tissue (decrease in cell size) compared to that of saline-injected controls. The mean interrenal cell size of fry receiving 2, 20 or 200 ugm. of cortisol acetate/gm.wt./day did not differ significantly. Dexamethasone injections similarly decreased mean interrenal cell size, although this decrease was only significant for the higher dosage of dexamethasone.

The mean epsilon cell nuclear diameters for the cortisol and dexamethasone injection experiments are presented in Figures 28 and 29, and Table III. In the cortisol experiment, mean diameters for uninjected and saline-injected controls did not differ significantly. However, mean diameters were smaller than the mean diameters of controls for all dosages

of cortisol acetate. This decreased nuclear size compared to saline-injected controls was very significant for fry treated with 2 and 200 ugm. cortisol acetate/gm.wt./day, but not for those receiving 20 ugm. cortisol acetate/gm.wt./day. Dexamethasone injections did not alter mean nuclear diameters of epsilon cells significantly.

For the various treatments, no consistent differences between males or females regarding mean interrenal nuclear diameters, numbers of inter-renal nuclei per defined area of interrenal tissue or epsilon cell nuclear diameters occurred.

Staining of Interrenal Cells of Saline, ACTH and Cortisol-injected Fry for RNA

The cytoplasm of interrenal cells of all fry injected with saline was faintly to moderately stained evenly with methylene blue. Nuclei and nucleoli were also stained (Fig. 33).

The cytoplasm of interrenal cells of fry treated with ACTH was intensely stained with methylene blue compared with interrenal cells of saline-injected fish (Fig. 35). All dosages of ACTH increased cytoplasmic basophilia, although higher dosages (0.2 and 0.4 I.U. ACTH/gm.wt./day) led to a more intense staining reaction than did 0.05 I.U. ACTH/gm.wt./day. The prominent nucleoli of interrenal cells of ACTH-treated fish were also more intensely stained than were the nucleoli of interrenal cells from control fish. Furthermore, interrenal cell nuclear membranes of ACTH-treated fish stained more intensely.

Cytoplasm of interrenal cells from cortisol-treated fish consistently stained faintly and often unevenly with methylene blue (Fig. 37). Nuclei stained moderately; the small nucleoli stained faintly.

Figure 33:   Interrenal cells from a coho fry treated with
              0.01 ml. saline/gm.wt./day for 7 days, stained
              for RNA. Note even staining of cytoplasm, and
              basophilia of nucleoli. Methylene blue. X1000.

Figure 34:   Interrenal cells from same fish as Figure 33.
              Section was treated with 1 N. HCl at 60°C. for
              10 min. prior to staining for RNA. Methylene
              blue. X1000.

Figure 35:   Interrenal cells from a coho fry treated with 0.2
              I.U. ACTH/gm.wt./day for 7 days, stained for RNA.
              Note intense basophilia of cytoplasm, prominent
              nucleoli and nuclear membranes compared with
              Figure 33. Methylene blue. X1000.

Figure 36:   Interrenal cells from same fish as Figure 35.
              Section was treated with 1 N. HCl at 60°C. for 10
              min. prior to staining for RNA. Methylene blue.
              X1000.

Figure 37:   Interrenal cells from a coho fry treated with 2
              ugm. cortisol acetate/gm.wt./day for 7 days,
              stained for RNA. Notice faint, irregular staining
              of cytoplasm. Methylene blue. X1000.

Figure 38:   Interrenal cells from same fish as Figure 37.
              Section was treated with 1 N. HCl at 60°C. for 10
              min. prior to staining for RNA. Methylene blue.
              X1000.

Since methylene blue specifically stains both DNA and RNA (Deitch, 1964), control slides for all treatments were hydrolyzed in 1 N. HCl at 60°C. for 10 minutes to remove RNA. Following extraction procedures with 1 N. HCl, interrenal cells from either saline, ACTH or cortisol-treated fish retained a comparable amount of residual basophilia (Figs. 34, 36 and 38). In all treatments, cytoplasm was very faintly blue. Nuclei and nucleoli decreased in stainability following extraction. Also, the intense basophilia of nuclear membranes of interrenal cells from ACTH-treated fish disappeared.

Effect of ACTH on Blood Cell Counts

The effect of ACTH injections on mean blood cell counts is presented in Table IV. Mean numbers of small lymphocytes per 1000 blood cells (S.L.'s/1000) for uninjected control fry and saline-injected controls did not differ significantly. However, all dosages of ACTH resulted in a marked and very significant decrease in S.L.'s/1000 compared to controls (Table IV and Fig. 27). No significant differences resulted from variation in dosage of hormone.

Mean numbers of small lymphocytes per 100 leucocytes (% S.L.'s) were very similar for both non-injected and saline-injected controls. All dosages of ACTH caused a fall in % S.L.'s from control values. This decrease was significant for all dosages except 0.1 I.U. ACTH/gm.wt./day.

Mean numbers of large lymphocytes per 1000 blood cells were negligible for both non-injected and saline-injected controls. Furthermore, large lymphocyte counts were unaffected by all dosages of ACTH. On the other hand, mean numbers of large lymphocytes per 100 leucocytes (% L.L.'s) were increased from controls by all dosages of ACTH. This increase was

TABLE IV

THE EFFECT OF ACTH-INJECTIONS ON BLOOD CELL COUNTS OF COHO FRY

No. of Fish	Treatment/ gm.wt./day for 7 Days	Water Temp. (°C.)	Mat. R.B.C.'s/ 1000	Imm. R.B.C.'s/ 1000	S.L.'s/ 1000	% S.L.'s	L.L.'s/ 1000	% L.L.'s	Nt.'s/ 1000	% Nt.'s	Throm.	Hct.
			←				MEAN $\pm$ S.E.					→
4	Uninjected controls	13.0	770.3 (25.4)	199.0 (25.6)	32.5 (4.3)	88.8 (8.4)	0.3 (0.3)	1.0 (0.7)	0.0	9.8 (7.2)	21.3 (3.6)	22.5 (2.3)
4	Saline- injected controls	13.0	857.8 (38.3)	97.5 (34.1)	43.3 (5.0)	90.3 (1.3)	0.0	2.0 (0.4)	1.5 (0.9)	7.8 (1.7)	25.8 (3.6)	22.0 (1.9)
4	0.05 I.U. ACTH	13.0	878.4 (20.1)	111.2 (19.3)	4.2 (2.5)	47.0 (13.5)	1.0 (0.5)	20.0 (5.3)	3.2 (0.8)	28.2 (6.2)	9.6 (3.7)	26.5 (1.2)
4	0.1 I.U. ACTH	13.0	677.3 (47.1)	315.9 (46.7)	4.0 (1.1)	77.0 (8.0)	0.5 (0.3)	7.8 (2.8)	2.3 (0.6)	14.5 (5.4)	9.8 (3.8)	21.5 (1.9)
4	0.2 I.U. ACTH	13.0	882.3 (24.4)	110.9 (22.1)	5.0 (2.1)	61.8 (5.8)	0.3 (0.3)	10.5 (1.9)	1.5 (0.6)	27.3 (4.1)	8.3 (1.3)	26.8 (3.0)
4	0.4 I.U. ACTH	13.0	861.0 (49.5)	127.0 (48.1)	4.5 (1.4)	45.0 (8.1)	0.5 (0.3)	10.8 (1.1)	6.8 (1.3)	40.3 (6.8)	13.8 (6.1)	12.2 (3.4)

significant for all dosages except 0.1 I.U. ACTH/gm.wt./day. Means for both controls did not differ significantly.

Mean numbers of neutrophils per 1000 blood cells (Nt.'s/1000) were small: 0 and 1.5 for non-injected and saline-injected controls respectively. Injections of 0.05, 0.1 or 0.2 I.U. ACTH/gm.wt. daily for 7 days did not affect mean Nt.'s/1000 significantly. However, the dosage of 0.4 I.U. ACTH/gm.wt. significantly increased mean Nt.'s/1000. Mean numbers of neutrophils per 100 leucocytes (% Nt.'s) also did not differ significantly between non-injected and saline-injected controls. However, all dosages of ACTH resulted in an increased mean % Nt. count compared with controls. This increase was significant for dosages of 0.05, 0.2 and 0.4 I.U. ACTH/gm.wt./day, but not for 0.1 I.U. ACTH/gm.wt./day.

Numbers of immature erythrocytes per 1000 blood cells were extremely variable from fish to fish in all treatments, as evidenced by the high S.E.'s (Table IV).

Mean hematocrit values were similar for both non-injected and saline-injected controls. Hematocrits for fry receiving different dosages of ACTH were variable, being larger for fry receiving 0.05 and 0.2 I.U. ACTH/gm.wt./day, but very significantly smaller for fry receiving the highest dosage of ACTH.

Mean numbers of thrombocytes amongst 1000 blood cells did not differ significantly between non-injected and saline-injected controls. However, all dosages of ACTH produced a marked decrease in thrombocyte counts (Fig. 27 and Table IV). This decrease was very significant for fry receiving 0.05, 0.1 and 0.2 I.U. ACTH/gm.wt./day, although not significant for those receiving 0.4 I.U. ACTH/gm.wt./day.

Effects of Cortisol and Dexamethasone on Blood Cell Counts

The effects of cortisol acetate and dexamethasone injections on mean blood cell counts are presented in Table V.

In the cortisol experiment, mean numbers of small lymphocytes per 1000 blood cells (S.L.'s/1000) did not differ significantly between non-injected and saline-injected controls. However, all dosages of cortisol resulted in a marked and significant decrease in mean S.L.'s/1000 (Fig. 28 and Table V). S.L.'s/1000 were negligible in fry receiving the highest dosage of cortisol. Similarly, no S.L.'s/1000 were found in smears of fry treated with either 1 or 10 ugm.'s of dexamethasone/gm.wt./day (Fig. 29 and Table V), although smears of saline-injected fry had a mean value of 7.8 S.L.'s/1000. Therefore, both cortisol and dexamethasone injections very markedly decreased the numbers of circulating small lymphocytes.

Mean numbers of small lymphocytes per 100 leucocytes (% S.L.'s) were similar for both saline-injected and non-injected controls. % S.L.'s for all dosages of cortisol were markedly and very significantly decreased when compared with saline-injected controls. Likewise, dexamethasone injections very significantly decreased mean % S.L.'s.

As was the case in the ACTH experiment, mean numbers of large lymphocytes per 1000 blood cells counted (L.L.'s/1000) were negligible for all controls in both the cortisol and dexamethasone experiments. Further-more, L.L.'s/1000 remained negligible in all fry treated with either cortisol or dexamethasone. On the other hand, although mean numbers of large lymphocytes per 100 leucocytes (% L.L.'s) were low (0.8 and 2.0) for non-injected and saline-injected controls in the cortisol experiment, % L.L.'s were increased for fry receiving all dosages of cortisol. This increase was very significant for fish administered 2 and 200 ugm.'s

TABLE V

THE EFFECT OF CORTISOL ACETATE-(FA) AND DEXAMETHASONE-(DEX) INJECTIONS ON BLOOD CELL COUNTS OF COHO FRY

No. of Fish	Treatment/ gm.wt./day for 7 Days	Water Temp. (°C.)	Mat. R.B.C.'s/ 1000	Imm. R.B.C.'s/ 1000	S.L.'s/ 1000	% S.L.'s	L.L.'s/ 1000	% L.L.'s	Nt.'s/ 1000	% Nt.'s	Throm.	Hct.
						MEAN ± S.E.						
4	Uninjected controls (FA)	10.0	850.8 (34.6)	131.0 (34.3)	15.5 (4.5)	87.5 (5.8)	0.0	0.8 (0.5)	0.3 (0.3)	11.5 (5.8)	11.0 (4.8)	26.4 (0.9)
4	Saline injected controls (FA)	10.0	920.0 (13.1)	66.8 (10.8)	12.3 (3.0)	83.3 (7.3)	0.0	2.0 (1.4)	1.0 (0.4)	14.8 (5.9)	11.8 (3.5)	25.3 (1.8)
5	2 ugm. FA	10.0	774.2 (63.7)	127.2 (45.3)	2.8 (1.2)	58.8 (7.7)	0.0	10.2 (2.6)	1.8 (0.9)	30.6 (5.7)	10.0 (3.8)	20.1 (1.5)
4	20 ugm. FA	10.0	936.8 (21.9)	59.3 (21.3)	2.0 (0.7)	42.0 (6.4)	0.0	11.3 (4.3)	2.0 (1.4)	46.8 (5.3)	8.5 (3.1)	26.8 (1.4)
5	200 ugm. FA	10.0	814.0 (31.7)	185.0 (31.4)	0.2 (0.2)	40.4 (7.0)	0.0	10.6 (2.9)	0.6 (0.2)	47.6 (4.6)	7.2 (1.3)	26.2 (1.9)
4	Saline-injected controls (DEX)	10.0	899.5 (46.9)	88.8 (47.3)	7.8 (1.2)	81.8 (2.1)	0.3 (0.3)	2.0 (0.8)	3.8 (1.0)	16.3 (2.6)	17.8 (2.3)	20.5 (1.5)
4	1 ugm. DEX	10.0	934.8 (14.1)	64.8 (14.4)	0.0	48.8 (4.2)	0.0	9.3 (2.3)	0.5 (0.3)	44.0 (3.0)	5.8 (2.3)	26.1 (1.2)
4	10 ugm. DEX	10.0	902.5 (22.9)	97.3 (37.0)	0.0	52.0 (4.1)	0.0	8.5 (0.5)	0.3 (0.3)	38.0 (4.3)	9.0 (3.0)	25.1 (2.6)

cortisol acetate/gm.wt./day, but not for those receiving 20 ugm.'s cortisol/ gm.wt./day. Similarly, dexamethasone injections resulted in a significant increase in mean % L.L.'s compared with saline-injected controls.

Mean numbers of neutrophils per 1000 blood cells (Nt.'s/1000) were small and not significantly different for both non-injected and saline- injected controls in the cortisol experiment. No dosages of cortisol significantly altered mean neutrophil counts from controls. However, mean numbers of neutrophils per 100 leucocytes (% Nt.'s), although not significantly different between non-injected and saline-injected controls, were markedly increased for all dosages of cortisol. This increase was very significant for fry treated with both 20 and 200 ugm.'s cortisol acetate/gm.wt./day.

In both experiments, mean numbers of immature erythrocytes per 1000 blood cells varied extensively from fish to fish for all treatments, as shown by the high S.E.'s for each treatment mean (Table V). No significant differences were found between the various treatments in both the cortisol and dexamethasone experiments.

In the cortisol experiment, mean hematocrit values were similar for both non-injected and saline-injected controls. Hematocrits were unaltered for fry receiving either 2 or 200 ugm.'s cortisol acetate/gm.wt./ day, although the mean hematocrit for those receiving 20 ugm.'s cortisol/ gm.wt./day was significantly decreased. On the other hand, hematocrits were higher for fry injected with dexamethasone when compared with saline- injected controls.

In the cortisol experiment, the mean numbers of thrombocytes amongst 1000 blood cells were similar for non-injected and saline-injected controls. Nevertheless, all dosages of cortisol acetate produced a decrease

(although not significant) in thrombocyte counts (Fig. 28 and Table V).
Similarly, both 1 and 10 ugm.'s of dexamethasone/gm.wt./day resulted in a
significant decrease in thrombocyte counts (Fig. 29 and Table V). These
results are comparable to the decrease in thrombocyte counts due to ACTH
injections.

In all of the injection experiments, no consistent differences in
mean blood cell counts were found when values for males and females from
each treatment were compared.

Correlation Coefficients of Variables Measured in the ACTH-Injection
Experiments

A correlation matrix of all variables measured from coho fry
involved in the ACTH-injection experiments is presented in Table VI.
Summary statistics (low and high measurements, average, standard deviation,
variance) for each variable are presented in Table I of Appendix II.

Interrenal nuclear diameters (I.N.D.'s) and numbers of interrenal
nuclei per defined area of interrenal tissue (No. Nu.'s) were negatively
correlated. Epsilon cell nuclear diameters (E.N.D.'s) were negatively
correlated (although not significantly) with I.N.D.'s, and correlated
positively (and significantly) with No. Nu.'s. I.N.D.'s were negatively
correlated with both numbers of small lymphocytes per 1000 blood cells
(S.L.'s/1000) and numbers of small lymphocytes per 100 leucocytes (% S.L.'s),
whereas No. Nu.'s were positively correlated with both variables. No
significant correlations existed between I.N.D.'s and numbers of large
lymphocytes per 1000 blood cells (L.L.'s/1000) or numbers of large
lymphocytes per 100 leucocytes (% L.L.'s),nor between either of these
variables and No. Nu.'s. However, interrenal nuclear diameters were very

TABLE VI

CORRELATION MATRIX OF VARIABLES MEASURED FROM COHO FRY INVOLVED IN ACTH EXPERIMENT (CONTROL + INJECTED FISH)

	Nt's/1000	% Nt's	Imm. R.B.C.'s/1000	THROM.	I.N.D.	No.Nu.	E.N.D.
Length (cm.)	0.494*	0.307	-0.642**	0.286	0.425	-0.273	-0.085
Weight (gm.)	0.486*	0.272	-0.617**	0.312	0.340	-0.198	-0.154
Hematocrit	-0.541*	-0.063	-0.194	-0.439	-0.321	0.177	0.010
S.L.'s/1000	-0.296	-0.589**	-0.016	0.767**	-0.696**	0.725**	0.636**
% S.L.'s	-0.597**	-0.973**	0.393	0.470*	-0.600**	0.564**	0.457*
L.L.'s/1000	0.208	0.098	-0.126	0.206	0.101	-0.026	0.048
% L.L.'s	0.342	0.692**	-0.309	-0.520*	0.293	-0.375	-0.644**
Nt's/1000	1.000	0.605**	-0.237	0.082	0.562**	-0.396	-0.339
% Nt's		1.000	-0.401	-0.405	0.612**	-0.511*	-0.318
Imm. R.B.C.'s/1000			1.000	-0.061	-0.108	-0.025	0.057
Thrombocytes				1.000	-0.189	0.447*	0.372
I.N.D.					1.000	-0.906**	-0.300
No.Nu.						1.000	0.465*
E.N.D.							1.000

*Significant at the 95 per cent level

**Significant at the 99 per cent level

Critical Absolute Values: 5%: 0.444
1%: 0.561

Nt's/1000: Neutrophils per 1000 blood cells

% Nt's: Neutrophils per 100 Leucocytes

Imm. R.B.C.'s/1000: Immature Erythrocytes per 1000 blood cells

THROM: Thrombocytes amongst 1000 blood cells

I.N.D.: Interrenal Nuclear Diameter

No.Nu.: Number of Interrenal Nuclei per defined Area of Interrenal Tissue

E.N.D.: Epsilon Cell Nuclear Diameter

TABLE VI

CORRELATION MATRIX OF VARIABLES MEASURED FROM COHO FRY INVOLVED IN ACTH EXPERIMENT (CONTROL + INJECTED FISH)

	LENGTH	WEIGHT	HEMATOCRIT	S.L.'s/1000	% S.L.'s	L.L.'s/1000	% L.L.'s
Length (cm.)	1.000	0.931**	-0.174	-0.122	-0.273	0.124	0.122
Weight (gm.)		1.000	-0.066	-0.173	-0.254	-0.009	0.140
Hematocrit			1.000	-0.129	0.004	-0.537*	0.241
S.L.'s/1000				1.000	0.625**	0.154	-0.537**
% S.L.'s					1.000	-0.037	-0.849**
L.L.'s/1000						1.000	-0.140
% L.L.'s							1.000
Nt's/1000							
% Nt's							
Imm. R.B.C.'s/1000							
Thrombocytes							
No.Nu.							
I.N.D. (microns)							
E.N.D. (microns)							

*Significant at the 95 per cent level   Critical Absolute Values:-   5% 0.444

**Significant at the 99 per cent level   1% 0.561

S.L.'s/1000:   Small Lymphocytes per 1000 blood cells

% S.L.'s: Small Lymphocytes per 100 Leucocytes

L.L.'s/1000:   Large Lymphocytes per 1000 blood cells

% L.L.'s: Large Lymphocytes per 100 Leucocytes

significantly positively correlated with both the numbers of neutrophils per 1000 blood cells (Nt.'s/1000) and numbers of neutrophils per 100 leucocytes (% Nt.'s). Numbers of thrombocytes amongst 1000 blood cells were negatively (but not significantly) correlated with I.N.D.'s, but positively and significantly correlated with No. Nu.'s. Neither I.N.D.'s nor No. Nu.'s were significantly correlated with lengths, weights, hematocrits or numbers of immature erythrocytes per 1000 blood cells.

Nuclear diameters of epsilon cells (E.N.D.'s) were positively correlated with S.L.'s/1000 and % S.L.'s. However, E.N.D.'s were not correlated significantly with either L.L.'s/1000 or Nt.'s/1000. E.N.D.'s were correlated positively with thrombocyte counts, although not significantly. No significant correlations existed between E.N.D.'s and either lengths or weights.

As well as being negatively correlated with I.N.D.'s and positively correlated with both No. Nu.'s and E.N.D.'s, numbers of small lymphocytes per 1000 blood cells were positively correlated with thrombocyte counts. S.L.'s/1000 were also positively correlated with % S.L.'s. However, S.L.'s/1000 were not correlated with either numbers of large lymphocytes or numbers of neutrophils per 1000 blood cells. No significant correlations existed between S.L.'s/1000 and either lengths or weights.

Numbers of small lymphocytes per 100 leucocytes (% S.L.'s) were negatively correlated with % L.L.'s, % Nt.'s and Nt.'s/1000, but not correlated with L.L.'s/1000. % S.L.'s and thrombocyte counts were positively correlated. L.L.'s/1000 and % L.L.'s were not correlated. However, Nt.'s/1000 and % Nt.'s were positively correlated.

Hematocrits were unrelated to all variables except numbers of neutrophils per 1000 blood cells, to which they exhibited a negative

correlation. Numbers of immature erythrocytes per 1000 blood cells were
negatively correlated with lengths and weights, but unrelated to all other
variables.

Correlation Coefficients of Variables Measured in the Cortisol and Dexamethasone Experiments

Correlation matrices for all variables measured from coho fry
involved in the cortisol and the dexamethasone injection experiments are
presented in Tables VII and VIII respectively. Summary statistics for each
variable are found in Tables II and III of Appendix II.

Interrenal nuclear diameters (I.N.D.'s) and numbers of interrenal
nuclei per defined area of interrenal tissue (No. Nu.'s) were negatively
correlated for fry from both cortisol and dexamethasone experiments. However,
correlation coefficients were not significant. Epsilon cell nuclear diameters
(E.N.D.'s) were positively correlated (although not significantly) with
I.N.D.'s, and very significantly negatively correlated with No. Nu.'s in
the cortisol experiment. On the other hand, correlation coefficients
between E.N.D.'s and I.N.D.'s or No. Nu.'s were not significant in fish
from the dexamethasone experiment.

I.N.D.'s were correlated positively (but not significantly) with
both numbers of small lymphocytes per 1000 blood cells (S.L.'s/1000) and
numbers of small lymphocytes per 100 leucocytes (% S.L.'s) in the cortisol
experiment, and positively and significantly with both variables in the
dexamethasone experiment. No. Nu.'s from the cortisol experiment were
negatively correlated with both S.L.'s/1000 and % S.L.'s. Likewise, No. Nu.'s
from the dexamethasone experiment were negatively correlated with S.L.'s/
1000 and % S.L.'s. Interrenal nuclear diameters were very significantly

TABLE VII

CORRELATION MATRIX OF VARIABLES MEASURED FROM COHO FRY INVOLVED
IN CORTISOL EXPERIMENT (UNINJECTED CONTROLS +
INJECTED FISH)

	LENGTH	WEIGHT	HEMATOCRIT	S.L.'s/1000	% S.L.'s	L.L.'s/1000	% L.L.'s
Length (cm.)	1.000	0.933**	0.101	0.162	0.239		-0.106
Weight (gm.)		1.000	0.095	0.294	0.356		-0.227
Hematocrit			1.000	0.135	0.110		0.003
S.L.'s/1000				1.000	0.737**		-0.496*
% S.L.'s					1.000		-0.783**
L.L.'s/1000							
% L.L.'s							1.000
Nt's/1000							
% Nt's							
Imm. R.B.C.'s/1000							
Thrombocytes							
I.N.D. (microns)							
No.Nu.							
E.N.D. (microns)							

*Significant at the 95 per cent level   Critical Absolute Values: 5%:   0.444
**Significant at the 99 per cent level   1%:   0.561

S.L.'s/1000:   Small Lymphocytes per 1000 blood cells   L.L.'s/1000:   Large Lymphocytes per 1000 blood cells
% S.L.'s: Small Lymphocytes per 100 Leucocytes   % L.L.'s: Large Lymphocytes per 100 Leucocytes

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TABLE VII
CORRELATION MATRIX OF VARIABLES MEASURED FROM COHO FRY INVOLVED
IN CORTISOL EXPERIMENT (UNINJECTED CONTROLS +
INJECTED FISH)

	Nt's/1000	% Nt's	Imm. R.B.C.'s/1000	THROM.	I.N.D.	No.Nu.	E.N.D.
Length (cm.)	-0.417	-0.261	-0.500*	0.164	-0.296	-0.245	0.154
Weight (gm.)	-0.351	-0.362	-0.534*	0.177	-0.154	-0.361	0.218
Hematocrit	-0.008	-0.131	-0.123	-0.035	-0.045	-0.176	0.111
S.L.'s/1000	-0.180	-0.744**	-0.072	0.567**	0.188	-0.577**	0.400
% S.L.'s	-0.110	-0.974**	-0.455*	0.300	0.019	-0.565**	0.467*
L.L.'s/1000							
% L.L.'s	-0.038	0.630**	0.340	-0.355	-0.025	0.471*	-0.189
Nt's/1000	1.000	0.195	-0.070	-0.032	0.242	0.183	-0.129
% Nt's		1.000	0.421	-0.202	-0.009	0.487*	-0.449*
Imm. R.B.C.'s/1000			1.000	-0.106	0.130	0.268	-0.357
Thrombocytes				1.000	0.210	-0.345	0.043
I.N.D.					1.000	-0.370	0.180
No.Nu.						1.000	-0.610**
E.N.D.							1.000

*Significant at the 95 per cent level   Critical Absolute Values: 5%:   0.444
**Significant at the 99 per cent level   1%:   0.561

Nt's/1000:   Neutrophils per 1000 blood cells   I.N.D.:   Interrenal Nuclear Diameter
% Nt's: Neutrophils per 100 Leucocytes   No.Nu.:   Number of Interrenal Nuclei per
Imm.R.B.C.'s/   Defined Area of Interrenal Tissue
   1000:   Immature Erythrocytes per 1000 blood cells   E.N.D.:   Epsilon Cell Nuclear Diameter
THROM.:   Thrombocytes amongst 1000 blood cells

TABLE VIII

CORRELATION MATRIX OF VARIABLES MEASURED FROM COHO FRY INVOLVED

IN DEXAMETHASONE EXPERIMENT

	LENGTH	WEIGHT	HEMATOCRIT	S.L.'s/1000	% S.L.'s	L.L.'s/1000	% L.L.'s
Length (cm.)	1.000	0.975**	0.108	0.121	0.190	-0.669*	-0.112
Weight (gm.)		1.000	0.150	0.162	0.306	-0.540	-0.235
Hematocrit			1.000	-0.637*	-0.319	-0.061	0.449
S.L.'s/1000				1.000	0.857**	0.341	-0.732**
% S.L.'s					1.000	0.515	-0.668*
L.L.'s/1000						1.000	-0.363
% L.L.'s							1.000
Nt's/1000							
% Nt's							
Imm. R.B.C.'s/1000							
Thrombocytes							
I.N.D.							
No.Nu.							
E.N.D.							

*Significant at the 95 per cent level   Critical Absolute Values: 5%: 0.576

**Significant at the 99 per cent level   1%: 0.708

S.L.'s/1000:   Small Lymphocytes per 1000 blood cells   L.L.'s/1000:   Large Lymphocytes per 1000 blood cells

% S.L.'s: Small Lymphocytes per 100 Leucocytes   % L.L.'s: Large Lymphocytes per 100 Leucocytes

	Nt.'s/1000	% Nt.'s	Imm.R.B.C.'s/1000	THROM.	I.N.D.	No.Nu.	E.N.D.
Length (cm.)	0.048	-0.212	-0.695*	0.066	-0.054	-0.383	0.009
Weight (gm.)	0.173	-0.271	-0.668*	0.140	-0.694	-0.446	0.082
Hematocrit	-0.327	0.185	-0.097	-0.346	-0.153	0.083	0.041
S.L.'s/1000	0.644*	-0.736**	-0.202	0.760**	0.655*	-0.669*	0.159
% S.L.'s	0.771**	-0.919**	-0.127	0.794**	0.691*	-0.744**	0.248
L.L.'s/1000	-0.556	-0.398	0.572	0.438	0.458	0.202	0.044
% L.L.'s	-0.752**	0.301	0.117	-0.363	-0.187	0.552	-0.048
Nt.'s/1000	1.000	-0.643*	-0.049	0.570	0.333	-0.503	-0.075
% Nt.'s		1.000	0.190	-0.801**	-0.789**	0.644*	-0.216
Imm. R.B.C.'s/1000			1.000	-0.089	0.010	0.346	-0.357
Thrombocytes				1.000	0.662*	-0.447	0.319
I.N.D.					1.000	-0.434	0.116
No.Nu.						1.000	-0.250
E.N.D.							1.000

*Significant at the 95 per cent level   Critical Absolute Values: 5% 0.576

**Significant at the 99 per cent level   1%: 0.708

Nt.'s/1000:   Neutrophils per 1000 blood cells   I.N.D.:   Interrenal Nuclear Diameter

% Nt.'s: Neutrophils per 100 Leucocytes   No.Nu.:   Number of Interrenal Nuclei per

Imm. R.B.C.'s/   Defined Area of Interrenal Tissue

   1000:   Immature Erythrocytes per 1000 blood cells   E.N.D.:   Epsilon Cell Nuclear Diameter

THROM: Thrombocytes amongst 1000 blood cells

negatively correlated with % Nt.'s in the dexamethasone experiment, although these variables were not significantly related in the cortisol experiment. Thrombocyte counts were positively correlated with I.N.D.'s and negatively (although not significantly) correlated with No. Nu.'s. Neither I.N.D.'s nor No. Nu.'s were significantly correlated with lengths, weights, hematocrits or numbers of immature erythrocytes per 1000 blood cells.

Epsilon cell nuclear diameters (E.N.D.'s) were positively (although not significantly) correlated with S.L.'s/1000, and significantly positively correlated with % S.L.'s in the cortisol experiment. Also, E.N.D.'s were negatively correlated with % Nt.'s. On the other hand, E.N.D.'s were not correlated significantly with L.L.'s/1000, % L.L.'s or Nt.'s/1000 in the cortisol experiment. Furthermore, no significant correlations existed between E.N.D.'s and thrombocyte counts, lengths or weights. In the dexamethasone experiment, nuclear diameters of epsilon cells were not significantly correlated with any variables.

Numbers of small lymphocytes per 1000 blood cells were positively correlated with both % S.L.'s and numbers of thrombocytes amongst 1000 blood cells in the cortisol and dexamethasone experiments. However, S.L.'s/1000 were not significantly correlated with L.L.'s/1000, or with Nt.'s/1000 for the cortisol experiment, although S.L.'s/1000 and Nt.'s/1000 were positively correlated in the dexamethasone experiment. No significant correlations existed between S.L.'s/1000 and either lengths or weights.

Hematocrits were not significantly correlated with any variables in the cortisol or dexamethasone experiments. Numbers of immature erythrocytes per 1000 blood cells (Imm.'s/1000) were negatively correlated with lengths and weights in both the cortisol and dexamethasone experiments. Notwithstanding a significant negative correlation between Imm.'s/1000

and % S.L.'s in the cortisol experiment, Imm.'s/1000 were not correlated

significantly with other variables in the cortisol or dexamethasone

experiments.

DISCUSSION

It is well established that the pituitary-adrenocortical axis of mammals is stimulated by numerous diverse environmental conditions referred to as "stressful" (Selye, 1950; Gorbman and Bern, 1962). Bush (1962) suggests that psychological "stresses" appear to be even more potent in activating the pituitary-adrenal axis of mammals than physical stimuli. Numerous mammalian species show increased mean adrenal size when subjected to increased population pressure, or "crowding" (Gorbman & Bern, 1962; Christian and Davis, 1964). Furthermore, several studies report a distinct relation between position in a dominance hierarchy and adrenocortical activity (Louch and Higginbotham, 1967). In addition, it has been observed that merely transferring rats from one cage to another resulted in a significant rise in plasma corticosteroid levels (Friedman and Ader, 1967). These and other reports indicate the sensitive response of the pituitary-adrenocortical axis of mammals to environmental alterations.

According to W.S. Hoar (1966), "....although the literature is still meagre, it is probable that the pituitary-adrenal system of the lower vertebrate is activated by 'stress' and that differences between the fishes or the amphibians and the mammals are differences in degree rather than kind". Effects which might be interpreted in terms of the "stress concept" have been noted among fishes under a variety of conditions. Marked hyperplasia of the interrenal tissue in Pacific salmon accompanying the spawning migration has been reported by Robertson and Wexler (1959). Chavin and Kovacevik (1961) have demonstrated histochemically rapid depletion with handling of ascorbic acid and cholesterol from the goldfish adrenocortical

cells. Furthermore, Chavin (1964) demonstrated that simply transferring goldfish from a given aquarium to another similar in all respects produced a hyperglycemia, requiring a four-day period of adjustment for the return to normal levels of serum glucose. Erickson (1967) reported a significant inverse correlation between the quantity of interrenal tissue and the number of aggressive actions of the sunfish, Lepomis gibosus in a dominance hierarchy study. Donaldson and McBride (1967) found that holding adult rainbow trout in an aquarium with the water level lowered to 2 - 3 cm. resulted in a significant increase in plasma cortisol levels in sham-hypophysectomized fish. Fagerlund (1967), after studying the effects of handling adult sockeye salmon on plasma cortisol levels, stated, "The results show that the pituitary-adrenal system of adult salmon is highly sensitive to various stressors and reacts with a manifold increase in the concentration of cortisol in peripheral plasma".

With the aforementioned considerations in mind, several precautions were taken in the present experiments to minimize a possible stimulation due to various conditions considered as potentially "stressful" of the pituitary-interrenal axis of juvenile coho salmon. In an attempt to simulate a more natural environment, both the holding tanks and experimental compartments were provided with gravel bottoms and artificial cover. Photocells enabled all tanks and compartments to receive a natural photoperiod. In all experiments, juvenile coho salmon transferred from Bertrand Creek to the laboratory were allowed several weeks for acclimatization to the stock tanks prior to treatment. Since juvenile coho salmon are extremely territorial fish which establish social hierarchies and display extensive aggressive behavior in both natural and laboratory environments (Chapman, 1962; Hartman, 1965; Mason and Chapman, 1965; Fraser, 1968), it was

considered necessary in all experiments reported here to place fish in

individual experimental compartments; thus eliminating any interactions

as reported in the study by Erickson (1967) with sunfish which could

possibly stimulate the interrenal gland. In addition, experimental

compartments were designed to provide fish with as large a territory as was

economically feasible. Furthermore, in consideration of Chavin's (1964)

study, all fish were held for seven days following transfer to experimental

compartments prior to treatment. It is not known whether or not these

precautions were sufficient or overly pretentious. However, it is interesting

to note that the mean size of interrenal cells and the mean diameter of

epsilon cell nuclei for the stock sample were significantly smaller than

values for either the non-injected or saline-injected control samples in

the ACTH experiment. Possibly the effect of transfer from stock tanks to

experimental compartments stimulated the pituitary-interrenal axis of

control fish somewhat.

Numerous investigations (Knobil et al., 1954; Miller, 1954;

Tonutti et al., 1954; Holley, 1965) with mammalian species have demonstrated

that administration of exogenous ACTH to intact or hypophysectomized

animals results in hypertrophy and hyperplasia of cells of the fascicular

and reticular zones of the adrenal cortex. This stimulation of the adrenal

cortex is accompanied by an increase in adrenal blood flow (Sapirstein and

Goldman, 1959; Staehelin et al., 1965; Maier and Staehelin, 1968). Further-

more, it is well-established that ACTH administration to intact or

hypophysectomized (but not adrenalectomized) mammals produces a marked

pycnosis and decrease in numbers of lymphocytes in all lymphoid tissues

(spleen, thymus, lymph nodes, Peyer's patches, etc.), concurrent with the

appearance of numerous edematous spaces within these structures (Dougherty

and White, 1945; Selye, 1950).

Rasquin (1951) first observed changes due to injections of mammalian ACTH in the histology of the head kidney and interrenal tissue of a teleost. She reported an increased vascularization of the head kidney of Astyanax mexicanus along with the appearance of numerous edematous spaces and a marked decrease in numbers of small lymphocytes two hours after ACTH administration. A marked hypertrophy and hyperplasia of the interrenal tissue, and an intimate association of the interrenal cells with the ramifications of the posterior cardinal vein were noted four hours after ACTH treatment. Krauter (1958) also reported an increased vascularization associated with hyperplastic interrenal tissue, and a marked decrease in numbers of lymphocytes in the head kidneys of Umbra krameri and Apomotis cyanellus following ACTH administration. Similar results were observed in this study for all dosages of ACTH, although vacuolation of the hematopoietic tissue was most pronounced with higher dosages. Whether this vacuolation or appearance of "edematous spaces" throughout the hematopoietic tissue can be attributed entirely to a pycnosis and decrease in number of lymphocytes within the head kidney is difficult to determine. According to G.W. Klontz (personal communication), urinary output of salmonids is greatly diminished by ACTH administration. The very significant decrease in hematocrit for coho fry receiving 0.4 I.U. ACTH/gm.wt./day may reflect a true edema of the hematopoietic tissue of these fish.

Several mammalian studies have utilized measurements of nuclear size as a criterion for assessing activity of adrenocortical cells. Miller (1954) and Tonutti et al. (1954) reported a significant increase in nuclear and nucleolar size of zona fasciculata cells following administration of ACTH to intact or hypophysectomized mice or rats. However, hypophysectomy

or administration of corticosteroids resulted in decreased nucleolar and nuclear size of these cells. Similar karyometric alterations were described for Rhesus monkeys (Knobil et al., 1954) and humans (Holley, 1965).

A limited number of studies using measurements of interrenal nuclear size as an index for quantitatively assessing interrenal activity have been made with several species of teleost fishes. Mammalian ACTH has been shown to produce a significant increase in size of interrenal cell nuclei when administered to intact or hypophysectomized fish (Krauter, 1958; Basu, Nandi and Bern, 1965; Hanke and Chester Jones, 1966; Hanke, 1966). Fagerlund, McBride and Donaldson (1968) demonstrated a very significant increase in mean interrenal nuclear diameters of adult sockeye salmon treated with mammalian ACTH. This increased nuclear diameter was associated with a marked increase in plasma cortisol concentration. Contrary to these findings, van Overbeeke and Ahsan (1966) noted that the interrenal cells of hypophysectomized Couesius plumbeus, although stimulated by salmon pituitary extracts, were unresponsive to injections of mammalian ACTH.

The findings reported here confirm the stimulatory effect of mammalian ACTH on teleostean interrenal cells. In addition to the extensive hyperplasia reported, both nuclear diameters of interrenal cells and interrenal cell sizes were uniformly and significantly increased by ACTH administration. Moreover, the response was dose-related. No previous reports have appeared on the dose-response relationship of interrenal cells to mammalian ACTH. The very significant positive correlation between interrenal nuclear diameter and cell size (measured as number of interrenal nuclei per defined area of interrenal tissue) firmly establishes that the entire interrenal cell hypertrophies under the influence of ACTH, rather than that the nucleus simply enlarges. Furthermore, although the indirect

measurement of interrenal cell size utilized in this investigation has not
been previously explored as a method for assessing increased interrenal cell
activity, cell size appears to provide a useful index of activity. In
addition, values representing cell size are far easier to determine than
the tedious measurements of nuclear diameters. As the very marked response
of the interrenal tissue reported herein is observed in intact rather than
hypophysectomized fish, the possibility that endogenous corticotropin
might play a role in the appearance of changes in the interrenal tissue
after administration of mammalian ACTH cannot be ignored.

In addition to the hypertrophy and hyperplasia of interrenal cells
in response to ACTH administration, a marked hypertrophy of the nucleoli
was observed in the present investigation. Also, the frequency of mitotic
figures increased considerably. Furthermore, both the cytoplasm and nucleoli
of interrenal cells of coho fry treated with ACTH demonstrated more tissue
basophilia due to RNA than those of control fish. Moreover, this increased
basophilia due to RNA of interrenal cells was more pronounced with higher
dosages of ACTH. Support for these observations is found in the literature.
Hanke and Chester Jones (1966) report that the interrenal cells of European
eels treated with ACTH have larger nuclei with very prominent nucleoli, and
increased tissue basophilia due to RNA. Fagerlund et al. (1968) observed
prominent nucleoli associated with increased interrenal nuclear diameters
and elevated plasma cortisol concentrations in adult sockeye salmon treated
with ACTH. Mitotic figures were detected more frequently in the interrenal
tissue of Umbra krameri (Krauter, 1958) and Carassius auratus (Krauter, 1958;
Oguri and Nace, 1966) treated with mammalian ACTH than in controls.

Since the experiments of Selye (1936) and those of Dougherty and
White (1943, 1945), the destructive effect of certain adrenocortical steroids

on lymphoid tissues of mammals has been generally recognized. However, little attention has been paid to the effect of corticosteroids on hematopoietic tissues of lower vertebrates. Krauter (1958) observed a marked vacuolation of the head kidney hematopoietic tissue of Umbra krameri when fish were injected with desoxycorticosterone acetate. Robertson et al. (1963) reported a moderate to marked reduction in lymphocyte numbers in the spleen and thymus of immature rainbow trout that received intraperitoneal implants of cortisol. In addition, Olivereau (1966) noted a depletion of cells of the lymphoid head kidney of Anguilla anguilla following cortisol administration.

In the present investigation, hematopoietic tissue of head kidneys from cortisol- or dexamethasone-treated coho fry was more disaggregated than that of controls, although the increased vacuolation was far less extreme than that observed in head kidneys of fish receiving ACTH. In addition, pycnosis and shrinkage of lymphocytes within the hematopoietic tissue was observed. Thus, the data reported here, as well as the meagre data available in the literature indicate that the lymphoid tissue of lower vertebrates resembles that of mammals in its response to administered adrenocorticosteroids and to ACTH. It is interesting to note that the increased vascularization associated with ACTH injections of head kidneys of coho fry was not observed following cortisol or dexamethasone administration. It has been postulated by Maier and Staehelin (1968) that the increase in adrenal blood flow induced by ACTH in mammals might be due to prostaglandins which could be formed from unsaturated fatty acids released by the cleavage of cholesterol esters. Whether the increase in vascularization of head kidneys observed is an independent effect of ACTH on the vascular system or, alternatively, results from increased steroidogenesis is not known. However, the possible advantages achieved by an increase in blood supply (more

extensive oxygen supply for increased energy demands, rapid means of conveying hormones synthesized) to a stimulated interrenal tissue are obvious. Conversely, cortisol or dexamethasone administration, resulting in interrenal atrophy, does not necessitate an increased blood supply to the head kidneys.

In the present experiments with coho fry, cortisol acetate, at all doses used, caused a very marked interrenal atrophy after 7 days administration. A significant reduction in mean interrenal nuclear diameter, and a very significant decrease in mean cell size occurred for all dosages when compared with controls. Accompanying these histometric indices of atrophy were observations of irregularly shaped, closely apposed interrenal nuclei with shrunken nucleoli, and an irregularly stained, vacuolated cell cytoplasm. In addition, numberous intercellular spaces within clusters of interrenal cells appeared, which were often invaded by blood cells. Interrenal response was similar for all dosages administered. This is not surprising in view of the fact that all doses of cortisol were well above the physiological plasma cortisol levels of salmonids (Fagerlund, 1967; Donaldson and McBride, 1967).

Although similar investigations are few, previous reports concerning the effects of exogenous corticosteroids on the interrenal tissue of teleost fishes concur with findings reported here. Spalding (cited in Chester Jones, 1957) reported cellular degeneration in the interrenal of brown trout following cortisone treatment. Krauter (1958) observed a significant decrease in interrenal nuclear volume in Carassius auratus following deoxycorticosterone acetate administration. In addition, Robertson et al. (1963) reported a general atrophy of adrenocortical cells when immature

rainbow trout were administered cortisol pellets. Furthermore, Basu et al. (1965) found that treatment of Tilapia mossambica with cortisol acetate caused a significant decrease in interrenal nuclear diameters. Olivereau (1966) observed that the nucleoli of interrenal cells of the European eel tended to disappear following cortisol treatment, and the mean nuclear diameter of interrenal cells was significantly reduced.

Dexamethasone, a synthetic corticosteroid which is more potent than natural steroids in suppressing adrenal function in mammals, has been used extensively to study pituitary-adrenal interrelationships. Administration of dexamethasone to mammals has been shown to suppress endogenous cortico-trophin secretion (Beaven et al., 1964; Purves and Sirett, 1965). Only two studies (Donaldson and McBride, 1967; Fagerlund and McBride, 1969) have reported utilizing dexamethasone in investigations concerning the pituitary-interrenal axis of fishes. In the present study, dexamethasone administered to coho salmon fry resulted in a very marked atrophy of interrenal tissue, similar to that due to cortisol injection. Mean interrenal nuclear diameters were very significantly decreased for fish receiving both doses of dexamethasone compared with saline-injected controls. Fagerlund and McBride (1969) demonstrated a similar decrease in mean nuclear diameters of interrenal cells of dexamethasone-treated adult sockeye salmon compared with peanut oil-injected controls. Furthermore, dexamethasone suppressed an elevation of plasma cortisol levels which was observed in uninjected controls following repeated blood collection. Donaldson and McBride (1967) reported that the injection of dexamethasone into sham-hypophysectomized rainbow trout resulted in a plasma cortisol level which was identical to that found in hypophysectomized fish.

Several investigations with various species of teleost fishes have

described a consistent atrophy of interrenal tissue following hypophysectomy (Chavin, 1956; Basu et al., 1965; van Overbeeke and Ahsan, 1966; Donaldson and McBride, 1967). This interrenal atrophy is prevented or reversed by administration of mammalian ACTH or teleost pituitary implants to hypophysectomized fish. Van Overbeeke and Ahsan's (1966) description of the interrenal tissue of hypophysectomized Couesius plumbeus (shrunken, irregularly shaped nuclei, inconspicuous nucleoli, non-homogeneous cytoplasm, intercellular spaces) closely resembles that described for coho fry in this investigation following either cortisol or dexamethasone administration. Although the results reported herein are consistent with the hypothesis that the administration of exogenous corticosteroids results in an inhibition of endogenous corticotrophin release from the pituitary, which leads to a subsequent atrophy of the interrenal tissue, the possibility that this degeneration might be due to a direct inhibitory effect of cortisol or dexamethasone on the interrenal tissue cannot be ignored. However, evidence from other investigations tends to negate this possibility. In hypophysectomized eels, cortisol had no further effect upon the regressed interrenal tissue (Chan et al., 1969). Furthermore, Donaldson and McBride (1967) showed that a dosage of 1 ugm./gm.wt. of dexamethasone reduced the circulating cortisol level in the rainbow trout; however, since exogenous ACTH could still stimulate the secretion of cortisol, this action was not a result of a direct action of the drug on the interrenal tissue. It is noteworthy that this dosage of dexamethasone was the lesser dosage used in this study. It would be interesting to determine whether atrophy of interrenal tissue would occur if fish were administered both cortisol or dexamethasone and ACTH simultaneously. Additional evidence for the postulated activity of cortisol on the pituitary of fish is provided by Purrot

and Sage (1967). Cortisol when added to the culture medium inhibited the release of ACTH from cultured goldfish pituitaries.

The epsilon cells, characteristically disposed in a sheet at the interface between the rostral pars distalis and the neurohypophysis, have been recognized morphologically in pituitaries of teleosts for many years (Ball and Baker, 1969); however, only recently has the role of ACTH-secretion been allocated to these cells. Identification of the epsilon cells has been aided by Olivereau's (1964) observation that these cells, normally chromophobic, are specifically stained with MacConaill's (1947) lead-hematoxylin. Identification of the corticotropin-producing cells in the pituitaries of mammals has depended to a large extent on the use of the anti-adrenal drug metopirone (SU 4885, Ciba). Liddle et al. (1959) found that metopirone inhibits 11β-hydroxylation of adrenal steroids and, via a negative-feedback mechanism, stimulates ACTH-release. This drug has also been used to identify the corticotrops in the European eel (Olivereau and Ball, 1963; Ball and Olivereau, 1966), Poecilia latipinna (Ball and Olivereau, 1966), sockeye salmon and rainbow trout (Fagerlund et al., 1968). In Poecilia and the eel, treatment with metopirone results in hypertrophy and hyperplasia of the interrenal cells, as well as a specific activation (hypertrophy of nucleoli, increased cytoplasmic RNA, degranulation of cytoplasm, appearance of mitotic figures) of only the epsilon cells in the pituitary (Ball and Olivereau, 1966). Metopirone treatment was characterized in both trout and salmon by a degranulation of the lead-hematoxylin-positive epsilon cells, and an increase in thickness of the palisade-like layer of epsilon cells together with hypertrophy of the interrenal cell nuclei and decreased plasma cortisol levels (Fagerlund et al., 1968). Olivereau and Olivereau (1968) further confirmed the role of the epsilon cell, by demonstrating that surgical removal of the

interrenal in _Anguilla_ resulted in a specific activation of the epsilon cells
(hypertrophy of nuclei, degranulation of cytoplasm). In addition, McKeown and
van Overbeeke (1969) recently further identified the epsilon cells as the
source of ACTH in the pituitary gland of the adult sockeye salmon by the
application of immunohistochemical techniques.

In this investigation, a palisade-like layer of lead-hematoxylin-positive
epsilon cells resembling those described for other salmonid species (Olivereau,
1964; van Overbeeke and McBride, 1967; Fagerlund et al., 1968) was observed
in the rostral pars distalis of coho fry, bordering the neurohypophysis. All
dosages of ACTH or cortisol acetate reduced mean nuclear diameters of epsilon
cells compared with non-injected or saline-injected controls. No previous
reports have appeared on the effects of ACTH or cortisol on mean nuclear
diameters of epsilon cells. However, unpublished work by Olivereau (quoted
in Olivereau, 1967) has shown that the epsilon cells of the eel are
inactivated by ACTH administration. Furthermore, Olivereau (1966) noted a
regression of the epsilon cells of the eel following cortisol treatment.
Fagerlund et al. (1968) was unable to observe any histological changes in
the epsilon cells of ACTH-treated sockeye salmon; however nuclear measure-
ments for each sample were not determined and compared.

Fagerlund and McBride (1969) reported a degranulation of the epsilon
cells of adult sockeye salmon following dexamethasone treatment. Similarly,
a degranulation of epsilon cells following ACTH, cortisol acetate or
dexamethasone injections was observed in this study. This diminished
granulation suggests that these treatments inhibited the production rather
than the release of ACTH, since one would expect that an inhibition of
ACTH release would result in an increased granulation. Indeed, a decreased
granulation could represent an increased secretory activity of the epsilon

cells; however, the cytology of the epsilon cells or interrenal cells in this and previous studies do not substantiate this.

Although one would expect the epsilon cells to respond similarly to either cortisol or dexamethasone injections, no significant decrease in mean diameters of epsilon cells was observed in dexamethasone-treated fish compared with saline-injected controls. This apparent lack of response might be accounted for by assuming a basal level of activity of epsilon cells in the saline-injected controls. The mean interrenal cell size of this sample was small, and interrenal tissue appeared quiescent. The degranulation of epsilon cells and atrophy of interrenal tissue observed in the dexamethasone-administered fry and not in saline-injected fish does support the hypothesis that dexamethasone treatment inhibited the production of ACTH by the epsilon cells. It would be worth-while to study the inhibitory effects of dexamethasone or cortisol on the epsilon cells and interrenal tissue of fry displaying a marked stimulation of the pituitary-interrenal axis.

Several investigators have reported alterations in the histology of the head kidney (Chavin, 1956) or cytology of interrenal cells (Mahon et al., 1962; Chavin, 1964; Basu et al., 1965) of teleosts, indicative of stimulation, following saline injections. Therefore, both saline-injected and non-injected controls were utilized in the ACTH- and cortisol-injection experiments. However, no histological or histometric differences in the epsilon cells, hematopoietic head kidney tissue or interrenal tissue were found between saline-injected or non-injected controls in either experiment. Thus, it is concluded that anaesthetizing fish followed by injection of small amounts of physiological saline daily for seven days does not noticeably stimulate the pituitary-interrenal axis of coho fry.

In addition to the well established lymphocytolytic action of

corticosteroids on the lymphoid tissue of mammals (Dougherty and White, 1943, 1945; Selye, 1950; Burton et al., 1967), administration of cortico-steroids or ACTH to mammals results in characteristic alterations in numbers of the different blood cell types in the peripheral circulation. A marked lymphopenia (decrease in numbers of circulating lymphocytes) is observed in mice, rabbits, rats and humans (Dougherty and White, 1944; Hills et al., 1948; Palmer et al., 1951) following treatment of intact animals with ACTH or corticosteroids (principally 11-oxycorticosteroids). Furthermore, ACTH or corticosteroid administration produces a characteristic eosinopenia (Hills et al., 1948; Speirs and Meyer, 1949; Palmer et al., 1951); and Selye (1950) has included changes in circulating eosinophils among the manifestations of the "general adaptation syndrome". In addition, ACTH administration results in a neutrophilia (increase in circulating neutrophils) in these animals; however, corticosteroid treatment is not followed by neutrophilia. Treatment of adrenalectomized mammals with ACTH produces a similar neutrophilia; however, no lymphopenia or eosinopenia results. Therefore it is concluded that, in mammals, the decrease in circulating lymphocytes or eosinophils is contingent upon the stimulation of a functionally competent adrenal cortex, whereas levels of circulating neutrophils, although altered by ACTH administration, are not affected by adrenocortical activity directly.

The leucocyte response of coho fry demonstrated in this study by injections of ACTH, cortisol or dexamethasone is comparable to that noted in mammals. A very marked decrease in circulating small lymphocytes due to administration of all dosages of either ACTH, cortisol acetate or dexamethasone resulted. This lymphopenic response, although consistent for all dosages of ACTH, was more pronounced with higher dosages of

cortisol acetate, while dexamethasone injections at either dosage almost
completely removed all lymphocytes from the peripheral circulation. To
the best of my knowledge, no previous reports have appeared on the lymphopenic
effect of dexamethasone. However, since the potency of this synthetic
steroid is believed to be 20-35 times that of cortisol, the response noted
here is not surprising. In addition to the lymphopenia observed, a marked
thrombocytopenia (decrease in circulating thrombocytes) resulted from
ACTH, cortisol acetate or dexamethasone administration. As in mammalian
studies, numbers of circulating neutrophils were unaffected by cortisol
or dexamethasone treatment; however, a definite neutrophilia was observed
with maximal dosages of ACTH. Since eosinophils are not present in the
circulation of coho fry, a comparison with the eosinopenic response of
mammals could not be made.

There is a surprising absence of comparative literature available
concerning alterations in numbers of circulating blood cell types of lower
vertebrates related to variations in the activity of the pituitary-
interrenal axis. The only comparable study available is that of Weinreb
(1958), who investigated the effects of single injections of ACTH,
cortisone or turpentine on circulating leucocyte counts of the rainbow
trout. Lymphopenia and thrombocytopenia resulted from "stress" (turpentine
injection), cortisone and ACTH injection, while neutrophilia followed
stress and ACTH injection, but not cortisone injection. Unlike these and
the present observations, Slicher (1961) reported that high doses of ACTH
or cortisol caused lymphocytosis (increase in circulating lymphocytes) in
intact Fundulus heteroclitus, two hours after injection. Similarly, sudden
immersion of Fundulus heteroclitus or Poecilia latipinna in ice water
caused a marked leucocytosis after two or four hours, which was pituitary-

dependent (Slicher et al., 1962; Slicher and Ball, 1962). This apparent
inconsistency in lymphocytic response is probably due to the discrepancies
in time involved between injection and autopsy. Thus, the lymphocytolytic
effect of corticosteroids observed in lymphatic tissue of fish could result
in a short-term lymphocytosis, followed by a lymphopenia with prolonged
exposure to corticosteroids. Additional support for the lymphopenic effect
of exogenous corticosteroids in lower vertebrates is provided by the work
of Bennett and Harbottle (1968). They found a significant lymphopenia in
tadpoles and adults of Rana catesbeiana 48 hours after the injection of
cortisol acetate, and in mature Rana pipiens 12-144 hours after cortisol
acetate-treatment.

The thrombocyte has been identified as a discrete blood cell type in
the circulation of fishes for many years (Jordan and Speidel, 1924; Jordan,
1938). Due to the cohesive nature of these cells (Jordan, 1938), and their
ability to radiate long cytoplasmic pseudopods which link with other thrombo-
cytes to form intricate thrombocytic networks (Gardner and Yevich, 1969), the
thrombocyte is believed to function in the blood clotting process. The
mammalian platelet, although functionally similar to the thrombocyte, is
derived from a cytoplasmic fragmentation of megakaryocytes, and is therefore
not homologous with the thrombocyte of fishes. In rats and humans, platelet
counts are apparently unaffected by ACTH administration (Greer and Brown,
1948), whereas a marked decrease in numbers of circulating thrombocytes
following ACTH or corticosteroid administration was observed here and in
rainbow trout (Weinreb, 1958). Additionally, numbers of thrombocytes
and numbers of small lymphocytes per thousand blood cells of all types
were very significantly positively correlated for all injection
experiments with coho fry. The site of thrombocyte production, although

not clearly established, is likely the hematopoietic head kidney tissue in salmonids (G.W. Klontz, personal communication). The similarities of small lymphocytes and immature thrombocytes suggests to some (Jordan, 1938; Catton, 1951) but not to others (Jakowska, 1956) that small lymphocytes are precursors of thrombocytes. Whatever their hematopoietic interrelationships, the responses of circulating small lymphocytes or thrombocytes to ACTH, cortisol or dexamethasone administration are similar, although lymphopenia is far more pronounced.

In this study, blood cells were counted in two ways: both routine differential white blood cell counts were performed, and one thousand blood cells (both erythrocytes and leucocytes) were counted and classified for all blood smears. The second approach provides a more "absolute" determination of the numbers of leucocyte cell types. However, the validity of this approach requires a relative constancy of numbers of erythrocytes. Since the leucocytes normally comprise only 1-3% of the total blood cell counts of juvenile coho salmon (calculated from data of Katz, 1949), a large variation in erythrocyte numbers is required to affect significantly the leucocyte counts. Furthermore, a variation of less than 20% was found when the largest and smallest mean red blood cell counts for monthly stream samples of juvenile coho salmon (Katz, 1949) were compared. Weinreb (1958) determined total red and white cell counts for rainbow trout under normal and experimental conditions. Since the erythrocyte count did not exhibit significant change, it was used as the basis for the differential leucocyte counts in a manner similar to the present investigation.

Glader et al. (1968) reported that pharmacological doses of cortisol acetate administered to intact rats depressed erythropoiesis somewhat,

whereas physiological doses were without effect. No consistent differences in mean numbers of immature erythrocytes per thousand blood cells was found in this study as a result of ACTH, cortisol or dexamethasone administration; thus this "absolute" method as a means for comparing differential leucocyte counts appears to be acceptable. Although differential counts made from a hemocytometer would be more absolute in regards to numbers of cells per cc. of blood, preliminary studies indicated the lack of feasibility of this approach, due to the fragile nature of thrombocytes and neutorophils, and the inability of properly differentiating blood cell types with the standard blood diluting solutions employed (Katz, 1949; Hesser, 1960; Slicher, 1961). Gardner and Yevich (1969) reported similar difficulties with the blood of cyprinodont fishes.

According to the differential white blood cell counts, injections of either ACTH, cortisol acetate or dexamethasone resulted in significant increases in numbers of large lymphocytes and numbers of neutrophils per hundred leucocytes. However, these were only _relative_ increases due to the decreases in percentages of lymphocytes. This is evident since numbers of large lymphocytes or neutrophils per thousand blood cells did not increase significantly due to these treatments, the only exception being the neutrophilia observed due to high dosages of ACTH. Furthermore, S.L.'s/1000 and % S.L.'s were very significantly positively correlated in all treatments, whereas L.L.'s/1000 and % L.L.'s were never correlated, and Nt.'s/1000 and % Nt.'s were not positively correlated in the cortisol and dexamethasone experiments, although Nt.'s/1000 and % Nt.'s were very significantly positively correlated in the ACTH experiment.

In conclusion, from the preceding data it is apparent that the interrenal tissue of coho fry is under pituitary control, and that a negative-feedback mechanism operates between the interrenal and the pituitary. Specific cytological and histometric alterations in the epsilon cells of the rostral pars distalis, correlated with modifications in activity of the interrenal cells, support the hypothesis that the epsilon cell is the source of ACTH. Furthermore, it is clearly evident that the pituitary-interrenal axis of juvenile coho salmon is capable of marked variations in activity, as displayed by the alterations observed in the histology and cytology of the head kidney, interrenal tissue and epsilon cells following administration of mammalian ACTH, cortisol acetate and dexamethasone to intact fry. In addition, the results reported herein indicate that measurements of interrenal and epsilon cell nuclei and estimates of interrenal cell size, together with observations of nucleolar prominence and mitotic figures are useful indices of pituitary-interrenal activity in juvenile coho salmon. As in mammals, changes in pituitary-adrenocortical activity of coho fry are reflected in characteristic alterations in numbers of specific circulating leucocytes. This inter-relationship between activity of the pituitary-interrenal axis and the numbers of specific leucocytes within the peripheral blood of juvenile coho salmon is supported by the many significant correlation coefficients present when variables serving as indices for pituitary-interrenal activity and differential leucocyte counts are compared.

SECTION II: THE ACTIVITY OF THE PITUITARY-INTERRENAL AXIS AND RELATED
              HEMATOLOGICAL CHANGES IN JUVENILE COHO CAPTURED SEASONALLY
              FROM BERTRAND CREEK

INTRODUCTION

The endocrine system in teleost fishes, as in other vertebrates, acts as a chemical link between the organism and its environment (Hoar, 1965). Variations in activity of the thyroid gland (Eales, 1965), gonads (Hoar, 1962) and pituitary gland (Hoar, 1966) occur in teleosts in association with seasonal changes in temperature or photoperiod. Alterations in activity of interrenal tissue of fishes have been associated with experimental modifications of environments (Mahon et al., 1962; Hane et al., 1966; Fagerlund, 1967); however, the response of the interrenal tissue of teleosts to seasonal variations in their natural environment has not been investigated.

The anadromous coho salmon, Oncorhynchus kisutch, occupies many of the coastal streams of British Columbia during the freshwater stage of its life cycle. Each fall, fertilized eggs are deposited in the gravel redds by spawning adults. Young coho emerge from the gravel in the spring, and take up a stream residence, occupying and defending territories (Chapman, 1962; Mason and Chapman, 1965; Hartman, 1965). Juvenile coho normally remain in streams for a period of approximately fourteen months, followed by seaward migration in May (Hartman, 1965; 1968). These sea-run salmon usually commence sexual maturation in their third summer of life, enter freshwater that fall, migrate to the approximate spawning site from which they emerged, spawn and die. Juvenile coho salmon are referred to as fry or "parr" during their year of stream residence. The term "parr"

distinguishes them from the "smolt" stage, which they usually enter in the first year of life just before emigration.

Coho fry are exposed to numerous environmental fluctuations (seasonal changes in water temperature, velocity, turbidity and dissolved oxygen content; variations in photoperiod; availability of food supply; intraspecific and interspecific competition) during their year of stream residence. It was therefore decided to ascertain whether or not the activity of the pituitary-interrenal axis of juvenile coho salmon varies seasonally, and whether this in turn leads to variations in differential leucocyte counts in association with possible variations in the pituitary-interrenal activity. In this manner, it was hoped that some insight might be provided into the normal role of the pituitary-interrenal axis of coho salmon during their first year of life within a stream environment.

MATERIAL AND METHODS

Juvenile coho were sampled seasonally from Bertrand Creek
(Langley, B.C.). The population of fry emerging from the gravel in the
spring of 1968 was sampled in April, June, September and December of 1968,
and in March and April of 1969 prior to seaward migration. Yearling coho
were also sampled in April 1968, concurrently with the sampling of newly-
emerged coho. Likewise, both recently-emerged and yearling coho were
sampled in April of 1969. An additional summer sample of coho fry was
obtained from Bertrand Creek in July, 1969. All fish were captured by
pole-seine.

The autopsy procedure performed at the Creek immediately after
capture of individual fish was identical to that described in Section I.
Histological techniques performed on blood smears, head kidneys and
pituitary glands in the laboratory are given in Section I. Techniques of
karyometry and statistical evaluation of results are also described in
Material and Methods of Section I.

RESULTS

Seasonal Histology of the Head Kidney and Interrenal Gland

All head kidneys of recently-emerged coho sampled on April 12, 1968 contained pronephric kidney tubules (KT) and glomeruli amongst the densely-packed hematopoietic tissue (Fig. 39). Small branches of the posterior cardinal vein and venous sinuses were scattered randomly throughout the head kidney. Several very small clusters of interrenal cells (IC) were observed within the hematopoietic tissue. These clusters were usually unassociated with venous branches. Interrenal cell nuclei were small and closely apposed to one another. Nucleoli were small, but obvious. Occasional clusters were observed with hypertrophied nuclei and prominent nucleoli. Cytoplasm of all interrenal cells was finely granular, and non-vacuolated (Fig. 40). Mitotic figures were observed infrequently.

Coho recently emerged from the gravel were also sampled on April 26, 1969. Pronephric tubules and glomeruli were present in all head kidneys. The hematopoietic tissue was much more vascularized than that of the April 19, 1968 sample of newly-emerged fish. Clusters of interrenal cells appeared larger than those of the April 19, 1968 sample, and were sometimes associated with venous sinuses. Occasional clusters with very hypertrophied nuclei were evident. Mitotic figures were observed infrequently.

Remnants of pronephric tubules were found in some head kidneys in the June 22, 1968 Bertrand Creek sample. Hematopoietic tissue was not excessively vascularized. The interrenal tissue consisted of small clusters of cells as discrete islets, or associated with small branches of the

Figure 39:   Head kidney of a recently-emerged coho fry, captured
             from Bertrand Creek in April 1968. Note sections of
             kidney tubules (KT) and small clusters of interrenal
             cells (IC) within the hematopoietic tissue (HT). Azan.
             X250.

Figure 40:   Higher magnification of a cluster of interrenal cells
             shown in Figure 39. Azan. X900.

Figure 41:   Head kidney of a coho fry captured from Bertrand Creek
             in June 1968, showing a cluster of interrenal cells
             (IC) within the hematopoietic tissue (HT). V: venous
             sinous. Azan. X250.

Figure 42:   Higher magnification of interrenal cells shown in Figure
             41. Note small, closely apposed nuclei with small or
             inconspicuous nucleoli. Azan. X900.

Figure 43:   Head kidney of a coho fry captured from Bertrand Creek
             in September 1968. Note cluster of interrenal cells
             (IC) surrounding a venous sinous. Azan. X250.

Figure 44:   Higher magnification of interrenal cells shown in
             Figure 43. Azan. X900.
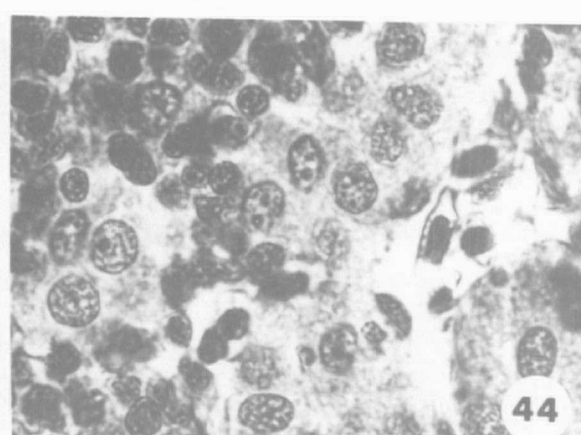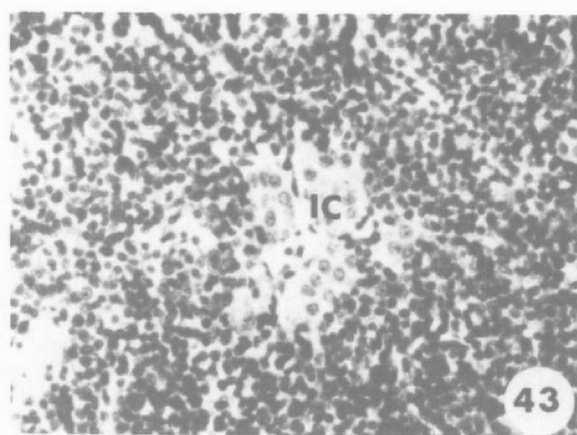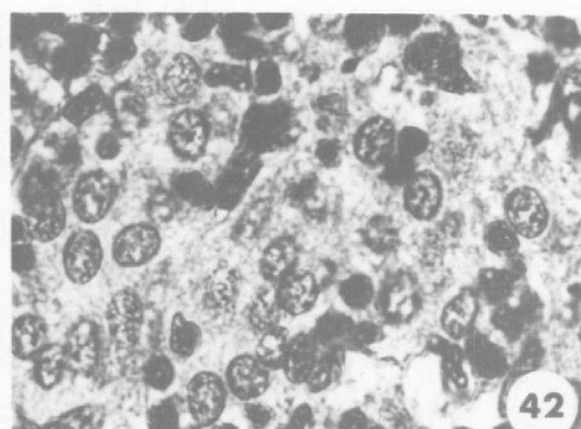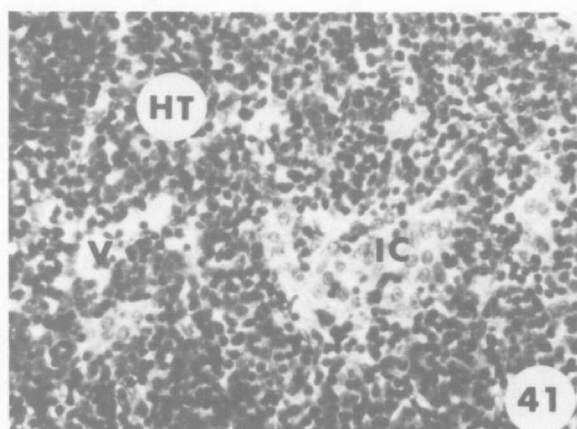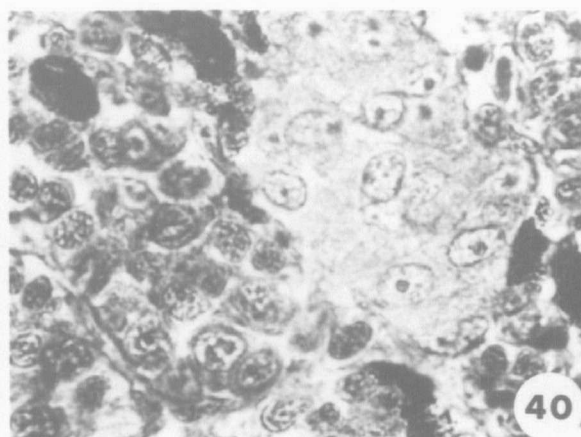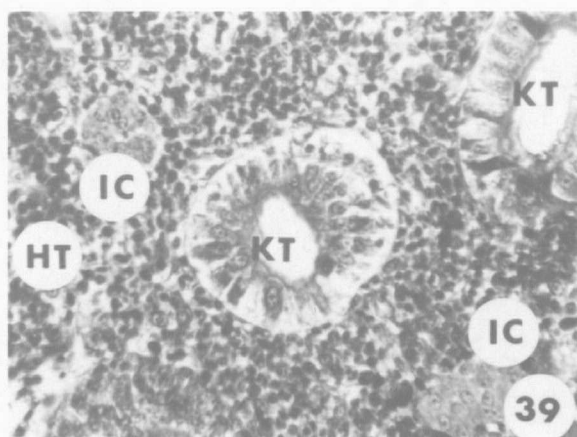
Figure 45:   Head kidney of a coho fry captured from Bertrand Creek
in December 1968. Extensive hyperplasia of interrenal
tissue has occurred compared with previous samples.
Many interrenal cells associated with branches of the
posterior cardinal vein (V). Azan. X250.

Figure 46:   Higher magnification of interrenal tissue shown in
Figure 45. Note marked hypertrophy of interrenal
cells. Nucleoli very prominent in all cells. Azan.
X900.

Figure 47:   Head kidney of a yearling coho captured from Bertrand
Creek in March 1969. Note interrenal tissue, with
frequent intercellular spaces, and invasion of
cluster of interrenal cells with blood cells. Azan.
X250.

Figure 48:   Higher magnification of interrenal cells shown in
Figure 47. Nuclei are often irregular in shape, with
small or inconspicuous nucleoli. Azan. X900.

Figure 49:   Head kidney of a yearling coho caught from Bertrand
Creek in April 1969. Note extensive hyperplasia of
interrenal tissue. Azan. X250.

Figure 50:   Higher magnification of interrenal cells shown in
Figure 49. All cells are hypertrophied, with
enlarged nuclei and very prominent nucleoli. Azan.
X900.

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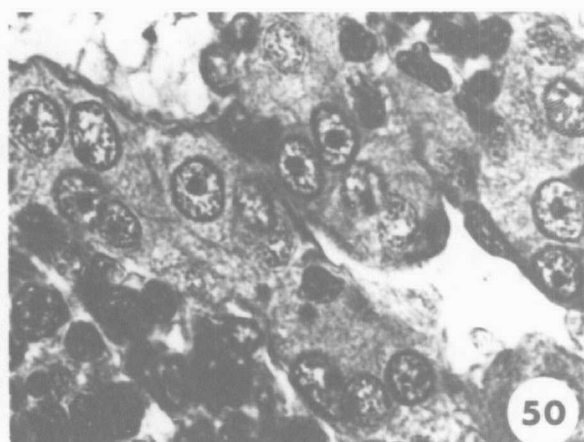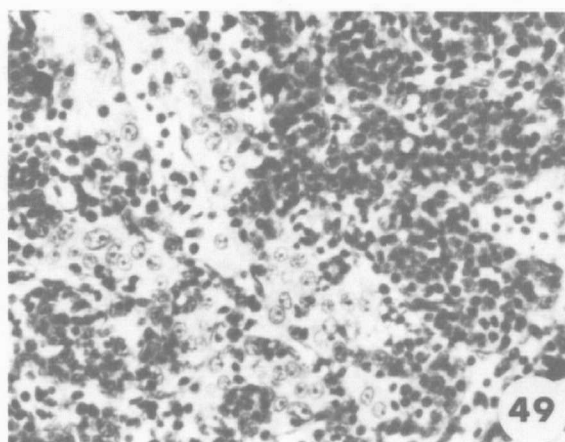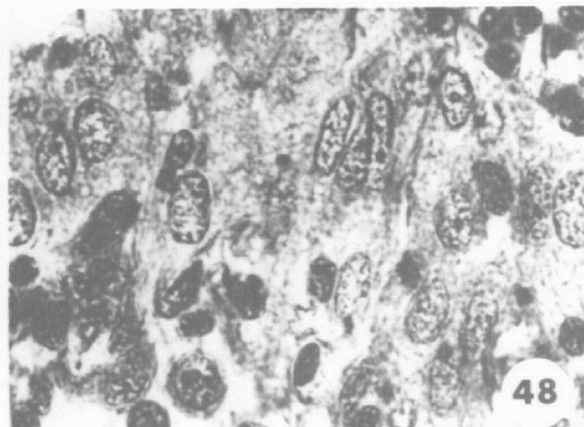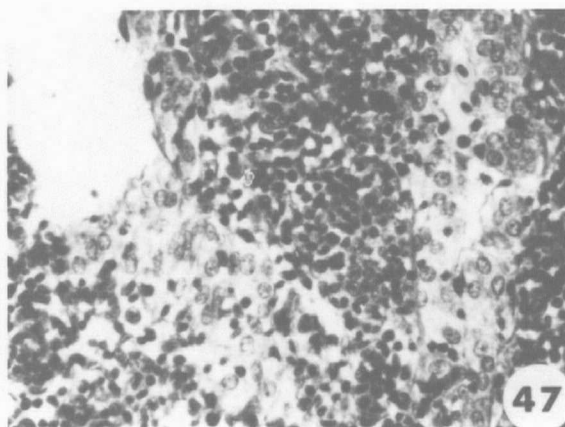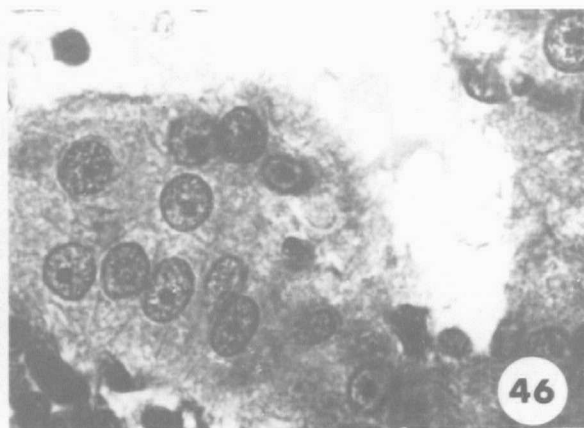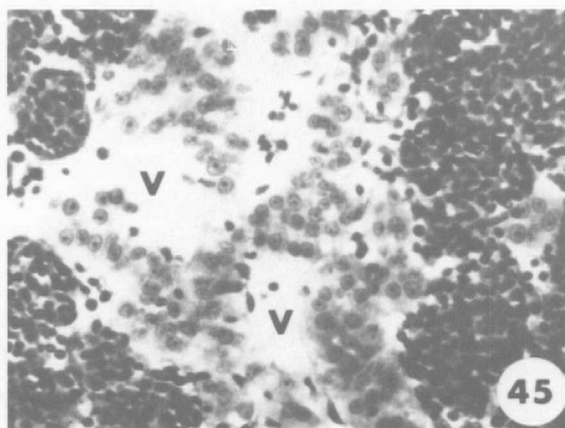
posterior cardinal vein or venous sinuses (Fig. 41). Interrenal nuclei were small and closely apposed to each other. Nucleoli were quite small, and often inconspicuous (Fig. 42). Cytoplasm of all cells was finely granular, and non-vacuolated. No clusters with hypertrophied nuclei were observed, and no mitotic figures were noticed within the interrenal tissue.

No pronephric tubules or glomeruli were present within the head kidneys of the July 26, 1969 or September 7, 1968 samples. Notwithstanding this, no differences were observed in the histology of the head kidney and interrenal tissue compared with the June 1968 sample (see Figs. 43 & 44).

The interrenal tissue of fry from the December 7, 1968 Bertrand Creek sample exhibited a marked hyperplasia compared with previous samples. Clusters of interrenal cells were much larger, and were very often associated with venous sinuses or small branches of the posterior cardinal vein (Fig. 45). Interrenal nuclei were hypertrophied, and contained very prominent nucleoli (Fig. 46). Mitotic figures were observed infrequently. A slight increase in vascularization of the head kidney existed when compared with previous stream samples.

Attempts were made to obtain samples from Bertrand Creek on February 15, 1969 (water temp. 4.5°C.), and on March 8, 1969 (water temp. 3.5°C.). However, no fish could be captured by pole seine during either attempt.

On March 29, 1969, a sample of yearling coho was obtained. Fish captured exhibited only a slight silvering indicative of smolting. Parr marks were still evident.

Head kidneys of the March, 1969 sample were more vascularized than those from the December 1968 sample. Hyperplasia of interrenal tissue was still very marked compared with summer or fall samples. However, the

cytology of interrenal cells from cluster to cluster varied considerably. Many clusters were observed with irregularly shaped, closely apposed nuclei, containing small nucleoli (Figs. 47 & 48). Cytoplasm of these cells was unevenly stained, and often vacuolated. Frequent intercellular spaces were observed. Blood cells frequently appeared to invade these clusters. In other clusters, nuclei were hypertrophied, and contained prominent nucleoli. Mitotic figures were very infrequently observed.

Yearling coho sampled on April 26, 1969 were very silver, unlike the earlier yearling sample. Parr marks were absent altogether, or only slightly discernible. Head kidneys of this sample were very highly vascularized. Interrenal tissue displayed extensive hyperplasia compared with summer and fall samples (Fig. 49). Nuclei of interrenal cells within all clusters were hypertrophied, with prominent nucleoli (Fig. 50). Cytoplasm was finely granular, evenly stained and not vacuolated. Mitotic figures were observed infrequently. Unlike the March 1969 sample, no signs of interrenal atrophy existed.

Yearling coho sampled on April 12, 1968 were moderately to very silver. Parr marks were faint, but still visible. Head kidneys of these fish were very well vascularized. Interrenal tissue was hyperplastic in all fish. The cytology of interrenal cells resembled that of the March 1969 sample for some fish, and that of the April 1969 sample for others. In several fish, interrenal nuclei were consistently small with inconspicuous nucleoli. Interrenal histology was extremely variable from fish to fish.

The mean diameters of the interrenal cell nuclei for all Bertrand Creek samples are presented in Figure 51 and Table IX. Using Scheffe's test for multiple comparisons, mean interrenal nuclear diameters for the April 1968 sample of coho recently-emerged from the gravel, the June 1968

TABLE IX

MEAN INTERRENAL NUCLEAR DIAMETERS (I.N.D.), NUMBER OF INTERRENAL NUCLEI
PER DEFINED AREA OF INTERRENAL TISSUE (NO.NU.), AND EPSILON CELL
NUCLEAR DIAMETERS (E.N.D.) OF JUVENILE COHO SALMON SAMPLED
SEASONALLY FROM BERTRAND CREEK

Number of Fish	Sample	Water Temp. (°C.)	I.N.D. (microns) Mean ± S.E.	NO.NU. Mean ± S.E.	E.N.D. (microns) Mean ± S.E.
6	April 12/68 Newly-emerged	8.5	5.84 ± 0.02	33.0 ± 1.0	-
6	April 26/69 Newly-emerged	8.5	6.09 ± 0.02	31.6 ± 0.7	-
12	June 22/68 Fry	14.5	5.82 ± 0.01	31.9 ± 0.8	5.00 ± 0.03 *(6)
6	July 26/69 Fry	17.5	5.67 ± 0.02	27.4 ± 0.7	-
6	September 7/68 Fry	10.5	5.76 ± 0.02	27.0 ± 0.6	5.19 ± 0.03 *(6)
6	December 7/68 Fry	4.0	6.31 ± 0.02	23.6 ± 1.1	5.55 ± 0.03 *(6)
7	March 29/69 Yearlings	10.0	6.37 ± 0.02	27.4 ± 0.6	-
6	April 26/69 Yearlings	10.0	6.41 ± 0.02	26.5 ± 1.4	-
12	April 12/68 Yearlings	8.5	6.15 ± 0.02	25.1 ± 0.5	5.56 ± 0.03 *(6)

*Sample size for Epsilon cell nuclear diameters

FIGURE 51:   Nuclear diameters of interrenal and epsilon cells, and

small-lymphocyte, thrombocyte and neutrophil counts in

juvenile coho salmon captured seasonally from Bertrand
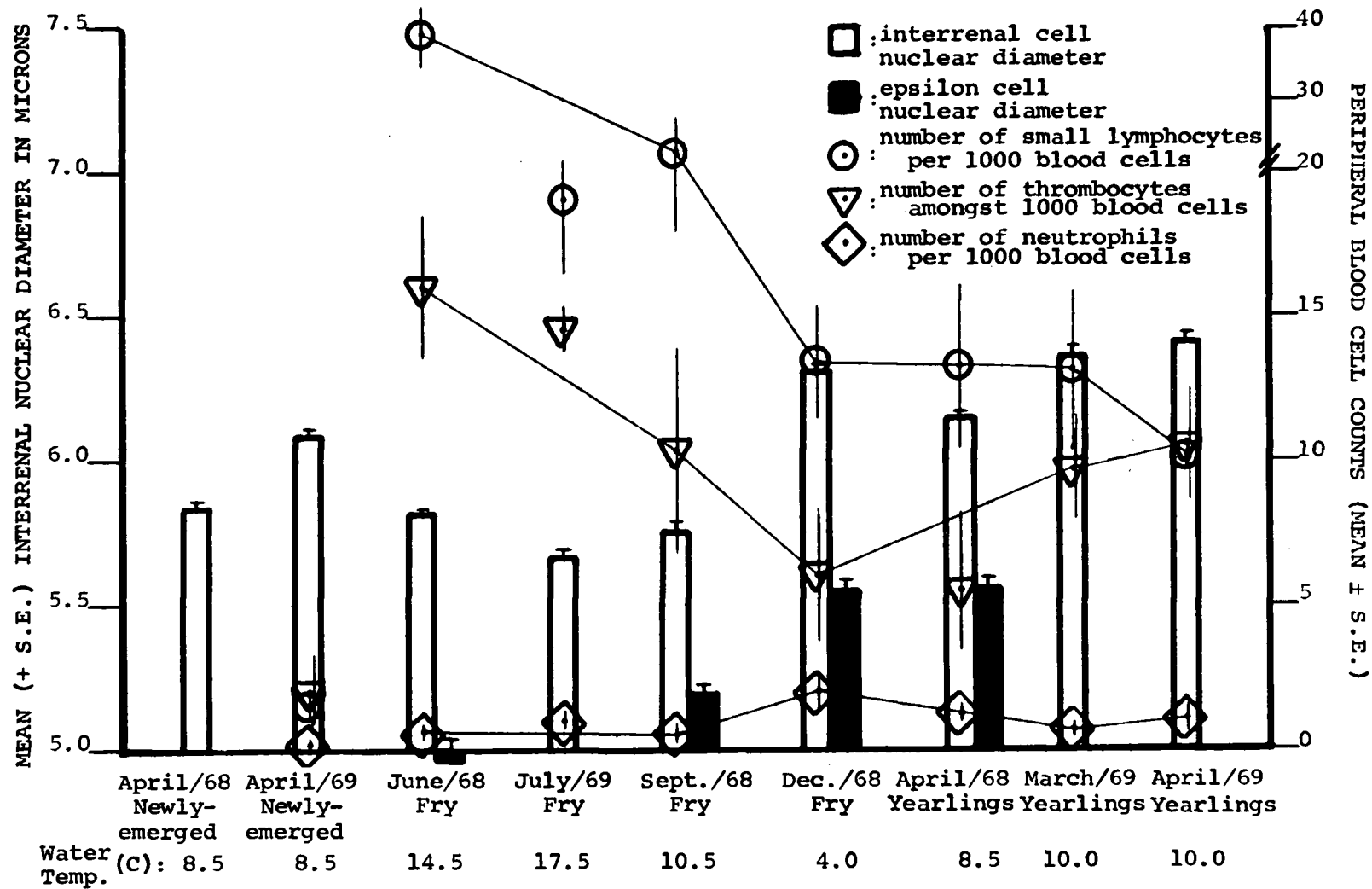
Creek.

FIGURE 52:   Interrenal cell size*in juvenile coho salmon captured
seasonally from Bertrand Creek.

MEAN (+ S.E.) NUMBER OF INTERRENAL
CELL NUCLEI PER DEFINED AREA**

samples from the population of coho
emerging from the gravel in Spring/68
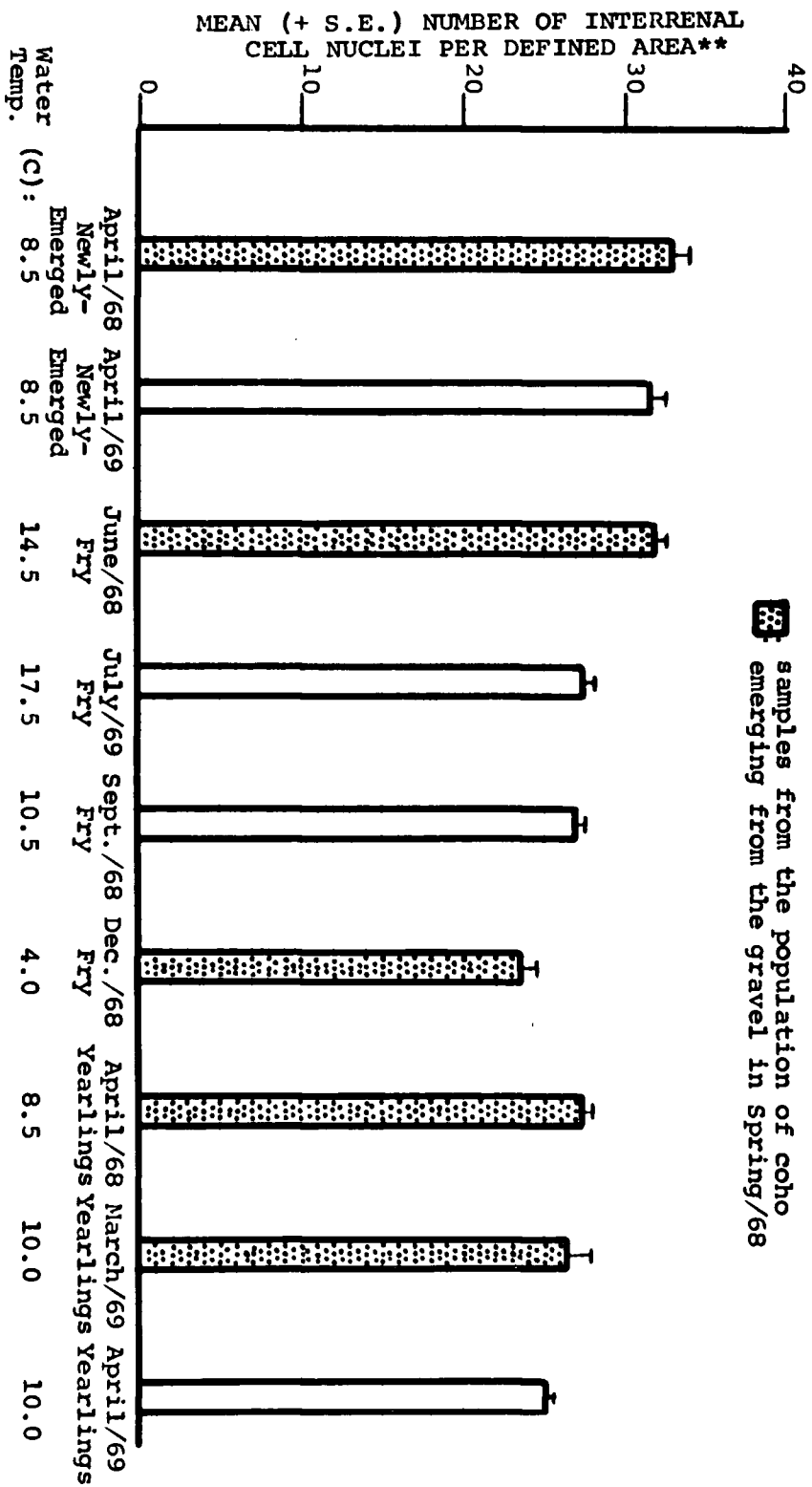
April/68 Newly-Emerged
April/69 Newly-Emerged
June/68 Fry
July/69 Fry
Sept./68 Fry
Dec./68 Fry
April/68 Yearlings
March/69 Yearlings
April/69 Yearlings

Water Temp. (C): 8.5 8.5 14.5 17.5 10.5 4.0 8.5 10.0 10.0

*Interrenal cell size is inversely related to the
number of interrenal cell nuclei per defined area.

**Defined area is 450 μ² of interrenal tissue.

and the September 1968 samples did not differ significantly from one another. All mean diameters for these samples were extremely small. However, the mean interrenal nuclear diameter for the December 1968 sample was very significantly increased. Although the mean I.N.D. of the March 1969 sample of yearling coho did not differ significantly from this sample, that of the April 1969 yearling sample was further increased significantly.

The mean I.N.D. of the April 1969 sample of newly-emerged coho was significantly increased over that of the April 1968 sample of newly-emerged fry. However, the mean diameter of the July 1969 sample did not differ significantly from that of the September 1968 sample. Mean I.N.D. of the April 1968 yearling coho sample was significantly less than both the December 1968 and yearling samples for the spring of 1969.

The mean interrenal cell size is represented in Figure 52 and Table IX. Mean interrenal cell sizes of the April 1968 and April 1969 newly-emerged samples and the June 1968 sample were similarly very small (large numbers of interrenal nuclei per defined area). Both July 1969 and September 1968 samples showed a comparable (but not significant) decrease in mean number of interrenal cell nuclei per defined area of interrenal tissue (increase in cell size). The December 1968 sample showed a further increase in mean interrenal cell size. Mean interrenal cell size of this winter sample was significantly larger than those of both the spring samples of recently-emerged fry and the June 1968 sample. Mean interrenal cell size of the December 1968 sample was also greater than mean cell sizes for all spring samples of yearling coho (although not significantly according to Scheffe's test). The April 1969 yearling sample had a slightly larger mean cell size when compared with that of the March 1969 sample.

Seasonal Histology of the Epsilon Cells in the Rostral Pars Distalis

The palisade-like layer of epsilon cells in the rostral pars distalis of the June 1968 sample was 2-3 cells in thickness. Elliptical nuclei were small and rather closely apposed, with inconspicuous nucleoli (Fig. 53). Cytoplasm was finely granular, and stained intensely with lead haematoxylin.

The palisade layer of epsilon cells in the September 1968 sample was also 2-3 cells thick. Cell nuclei were elliptical with inconspicuous nucleoli (Fig. 54). Cytoplasm was very finely granular, and moderately positive to lead hematoxylin.

The epsilon cells of the December 1968 Bertrand Creek sample appeared larger than those of the previous sample, with enlarged nuclei (Fig. 55). Nucleoli were obvious in many nuclei. Cytoplasm was very finely granular, and stained moderately. The palisade layer was 2-4 cells thick.

In the April 1968 sample of yearling coho, the epsilon cells usually resembled those of the December 1968 sample, with enlarged nuclei, and nucleoli evident (Fig. 56). However, in a few yearlings, nucleoli were always inconspicuous, and nuclei appeared somewhat smaller.

Mean epsilon cell nuclear diameters for the June 1968, September 1968, December 1968 and April 1968 samples are presented in Figure 51 and Table IX. The mean diameter for the June 1968 fry sample was very small (5.00 microns). However, the mean diameter of epsilon cell nuclei for the September 1968 sample was significantly increased. A further and very significant increase in mean epsilon cell nuclear diameters compared with June or September 1968 samples occurred in both the winter and the spring sample of yearling coho. Mean epsilon cell nuclear diameters for December

Figure 53: The palisade layer of the epsilon cells in the
            rostral pars distalis of a pituitary from a coho
            fry caught in June 1968. Nuclei are small, with
            inconspicuous nucleoli. Note the intensive
            staining of the cytoplasm of these cells. NH:
            neurohypophysis. Lead hematoxylin. X900.

Figure 54: The palisade layer of lead hematoxylin-positive
            cells in the rostral pars distalis of a coho fry
            caught in September 1968. Nuclei are small, with
            inconspicuous nucleoli. Cytoplasm stains moderately.
            NH: neurohypophysis. Lead hematoxylin. X900.

Figure 55: The epsilon cell layer in the rostral pars distalis
            of a coho fry captured from Bertrand Creek in
            December 1968. Epsilon cell nuclei are enlarged,
            with nucleoli often readily discernible.
            NH: neurohypophysis. Lead hematoxylin. X900.

Figure 56: The palisading layer of lead hematoxylin-positive
            cells in the rostral region of a pituitary from a
            yearling coho caught in April 1968. Nuclei appear
            enlarged compared with summer or fall samples, with
            nucleoli evident. NH: neurohypophysis. Lead
            hematoxylin. X900.

NH

NH

NH

NH

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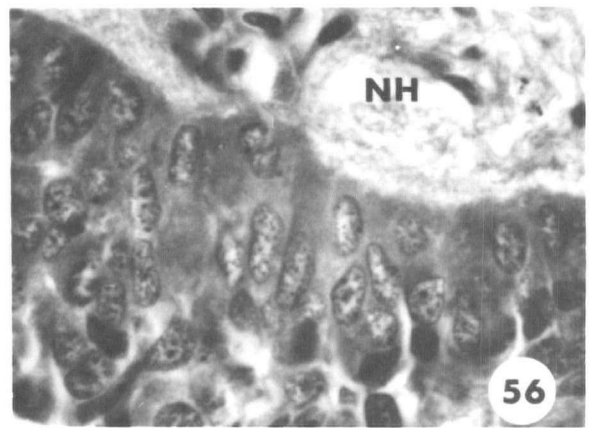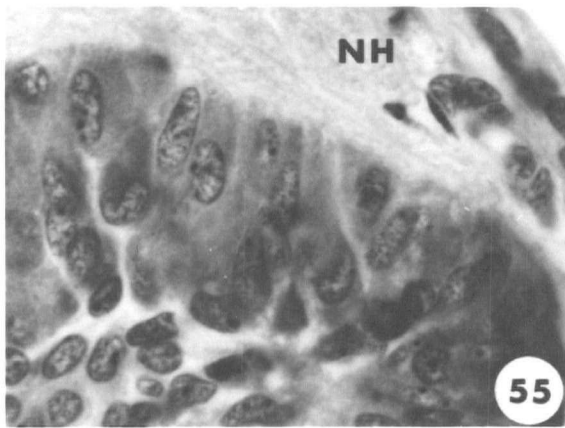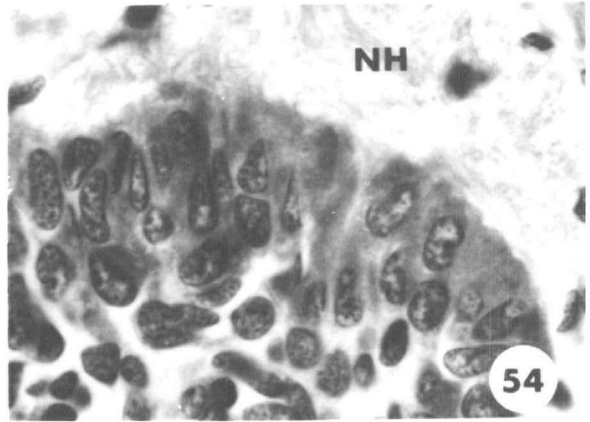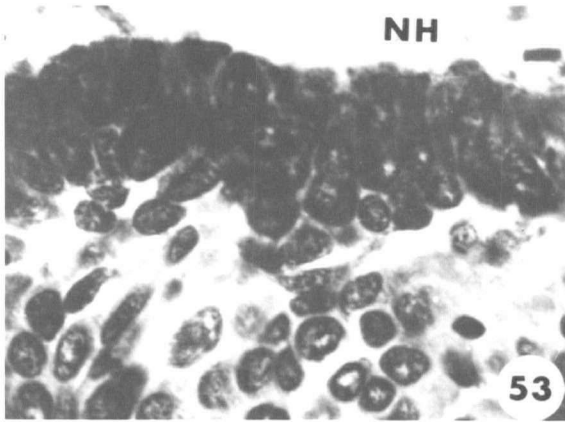
1968 and April 1968 yearling samples did not differ significantly.

Mean Blood Cell Counts for Seasonal Samples

The mean blood cell counts for seasonal samples of juvenile coho from Bertrand Creek are presented in Table X. Mean numbers of small lymphocytes per 1000 blood cells (S.L.'s/1000) were highest for summer and fall samples. However, a marked and very significant decrease in S.L.'s/1000 occurred in the December 1968 sample (Table X and Fig. 51) compared with the June 1968 sample. This decrease in numbers of circulating small lymphocytes persisted in the March 1969 yearling sample, with a further (although not significant) decrease in S.L.'s/1000 in the April 1969 sample of yearling coho. Mean S.L.'s/1000 for the April 1968 yearling sample was very similar to that for the March 1969 yearling sample. The mean number of small lymphocytes per 1000 blood cells for the April 1969 sample of fry recently emerged from the gravel was extremely low compared with all other samples.

Mean numbers of small lymphocytes per 100 leucocytes (% S.L.'s) were highest for both summer samples compared with other seasonal samples. % S.L.'s were slightly (although not significantly) decreased in the September 1968 sample. % S.L.'s for the December 1968 sample were significantly decreased from summer values. % S.L.'s of March 1969 and April 1969 samples of yearling coho were again increased (although not significantly) compared with % S.L.'s for the winter sample. The mean number of small lymphocytes per 100 leucocytes for the April 1969 sample of newly-emerged fry was significantly lower than the % S.L.'s for all other samples.

Mean numbers of large lymphocytes per 1000 blood cells (L.L.'s/1000) were negligible for all stream samples. Mean numbers of large

TABLE X

BLOOD CELL COUNTS OF JUVENILE COHO SALMON SAMPLED
SEASONALLY FROM BERTRAND CREEK

No. of Fish	Treatment/ gm.wt./day for 7 Days	Water Temp. (°C.)	Mat. R.B.C.'s/ 1000	Imm. R.B.C.'s/ 1000	S.L.'s/ 1000	% S.L.'s	L.L.'s/ 1000	% L.L.'s	Nt.'s/ 1000	% Nt.'s	Throm.
						MEAN ± S.E.					
6	Apr. 26/69 Bert. Cr. Newly-Emerged	8.5	926.0 (10.9)	73.8 (10.9)	1.5 (0.6)	68.7 (5.1)	0.2 (0.2)	3.0 (1.3)	0.2 (0.2)	28.5 (4.3)	2.0 (1.3)
12	June 22/68 Bert. Cr. Fry	14.5	941.5 (7.7)	17.0 (4.9)	38.9 (4.5)	95.8 (0.9)	0.4 (0.2)	1.1 (0.5)	0.6 (0.2)	2.6 (0.7)	16.0 (2.5)
6	July 26/69 Bert. Cr. Fry	17.5	944.2 (20.2)	35.8 (18.4)	19.0 (2.7)	95.5 (1.1)	0.0	0.2 (0.2)	1.0 (0.3)	4.3 (1.0)	14.5 (0.8)
6	Sept. 7/68 Bert. Cr. Fry	10.5	959.2 (3.9)	17.5 (4.2)	22.7 (5.0)	90.8 (2.8)	0.2 (0.2)	2.0 (0.9)	0.5 (0.2)	7.2 (2.2)	10.3 (3.6)
6	Dec. 7/68 Bert. Cr. Fry	4.0	943.7 (17.1)	42.2 (16.0)	13.3 (2.0)	83.7 (3.4)	0.3 (0.2)	5.0 (1.0)	2.0 (0.7)	11.3 (3.0)	6.0 (2.3)
12	Apr. 12/68 Bert. Cr. Yearlings	8.5	840.8 (17.9)	144.4 (16.6)	13.3 (3.0)	85.3 (3.5)	0.3 (0.1)	5.2 (1.1)	1.2 (0.3)	8.8 (2.8)	5.5 (2.1)
7	March 29/69 Bert. Cr. Yearlings	10.0	913.6 (10.2)	72.6 (11.5)	13.1 (2.8)	92.0 (1.5)	0.1 (0.1)	0.6 (0.4)	0.6 (0.2)	7.4 (1.5)	9.7 (1.9)
6	Apr. 26/69 Bert. Cr. Yearlings	10.0	957.0 (7.4)	31.7 (7.4)	10.2 (0.5)	89.8 (1.1)	0.2 (0.2)	2.0 (0.5)	1.0 (0.5)	8.2 (1.1)	10.5 (2.0)

lymphocytes per 100 leucocytes (% L.L.'s) were very low for summer samples and for the September 1968 samples. The mean number of large lymphocytes per 100 leucocytes for the December 1968 sample was slightly but significantly increased compared with the % L.L.'s of summer samples. % L.L.'s for the March 1969 sample of yearling coho were significantly decreased from the December 1968 value. The mean number of large lymphocytes per 100 leucocytes for the April 1969 sample of newly-emerged fry did not differ significantly from other samples.

The mean numbers of neutrophils per 1000 blood cells (Nt.'s/1000) were small for all samples, and did not differ significantly for any comparison. However, the December 1968 winter sample had the highest mean value. Mean numbers of neutrophils per 100 leucocytes (% Nt.'s) were lowest for summer samples. % Nt.'s increased slightly in the September 1968 sample compared with summer samples. A further (although not significant) increase in % Nt.'s was found in the December 1968 sample. Values for spring samples of yearling coho decreased again compared with the winter sample. The mean number of neutrophils per 100 leucocytes for the April 1969 sample of newly-emerged fry was very significantly greater than % Nt.'s for all other samples.

As was observed in the injection experiments, numbers of immature erythrocytes per 1000 blood cells (Imm.'s/1000) were extremely variable from fish to fish in all samples, as evidenced by the high S.E.'s. Imm.'s/1000 for summer and fall samples were relatively low compared with the December 1968 sample, and the March 1969 and April 1968 samples of yearling coho. The mean number of immature erythrocytes per 1000 blood cells was high for the April 1969 sample of recently-emerged coho fry compared with summer and fall samples.

Mean numbers of thrombocytes amongst 1000 blood cells were highest for summer samples. Thrombocyte counts were decreased (although not significantly) in the September 1968 sample, and significantly decreased in the December 1968 sample compared with values for summer samples. Thrombocyte counts for the March 1969 and April 1969 samples of yearling coho again increased relative to the winter sample (Table X and Fig. 51). The mean number of thrombocytes for the April 1969 sample of recently-emerged coho was extremely low compared with all other stream samples.

No consistent differences in mean blood cell counts, I.N.D.'s, No. Nu.'s or E.N.D.'s due to sex were found when values for males and females from each sample were compared.

Correlation Coefficients of Variables Measured in the Seasonal Samples

A correlation matrix for all variables measured from juvenile coho salmon sampled seasonally from Bertrand Creek is presented in Table XI. Summary statistics (low and high measurements, average, standard deviation, variance) for each variable are presented in Table IV of Appendix II.

Interrenal nuclear diameters (I.N.D.'s) and numbers of interrenal nuclei per defined area of interrenal tissue (No. Nu.'s) were negatively correlated. Epsilon cell nuclear diameters (E.N.D.'s) were positively correlated with I.N.D.'s and negatively correlated with No. Nu.'s. On the other hand, I.N.D.'s were negatively correlated with numbers of small lymphocytes per 1000 blood cells (S.L.'s/1000) whereas No. Nu.'s were positively correlated with S.L.'s/1000. However, neither I.N.D.'s nor No. Nu.'s were significantly correlated with numbers of large lymphocytes per 1000 blood cells (L.L.'s/1000) or numbers of neutrophils per 1000 blood cells (Nt.'s/1000). Interrenal nuclear diameters were negatively (although

TABLE XI

CORRELATION MATRIX OF VARIABLES MEASURED FROM JUVENILE COHO SALMON
SAMPLED SEASONALLY FROM BERTRAND CREEK

	Nt.'s/1000	% Nt.'s	Imm. R.B.C.'s/1000	THROM.	I.N.D.	No.Nu.	**E.N.D.
Length (cm.)	0.221	-0.355*	0.243	0.164	0.532**	-0.571**	0.497*
Weight (gm.)	0.182	-0.258	0.267	0.143	0.591**	-0.508**	0.461
Temperature	-0.240	-0.418**	-0.394**	0.491**	-0.544**	0.501**	-0.708**
S.L.'s/1000	-0.028	-0.527**	-0.275	0.480**	-0.477**	0.336*	-0.507*
% S.L.'s	0.131	-0.951**	-0.325*	0.489**	-0.186	0.100	-0.609**
L.L.'s/1000	0.001	-0.173	-0.070	0.151	-0.007	-0.034	-0.304
% L.L.'s	-0.010	0.284	0.328*	-0.313*	0.312*	-0.323*	0.401
Nt.'s/1000	1.000	0.038	-0.079	0.165	0.090	-0.112	0.296
% Nt.'s		1.000	0.237	-0.458**	0.128	0.006	0.577**
Imm. R.B.C.'s/1000			1.000	-0.190	0.304*	-0.343*	0.507*
Thrombocytes				1.000	-0.174	0.089	-0.376
I.N.D.					1.000	-0.538**	0.658**
No.Nu.						1.000	-0.721**
E.N.D.							1.000

*Significant at the 95 per cent level
**Significant at the 99 per cent level

Critical Absolute Values: 5%: 0.288
1%: 0.372

Nt.'s/1000: Neutrophils per 1000 blood cells
% Nt.'s: Neutrophils per 100 Leucocytes
Imm. R.B.C.'s/1000: Immature Erythrocytes per 1000 blood cells
THROM: Thrombocytes amongst 1000 blood cells

I.N.D.: Interrenal Nuclear Diameter
No.Nu.: Number of Interrenal Nuclei per Defined Area of Interrenal Tissue
E.N.D.: Epsilon Cell Nuclear Diameter
** Critical Absolute Values for E.N.D.'s: 5%: 0.413
1%: 0.526

TABLE XI
CORRELATION MATRIX OF VARIABLES MEASURED FROM JUVENILE COHO SALMON
SAMPLED SEASONALLY FROM BERTRAND CREEK

	LENGTH	WEIGHT	TEMPERATURE	S.L.'s/1000	% S.L.'s	L.L.'s/1000	% L.L.'s
Length (cm.)	1.000	0.961**	-0.042	-0.043	0.294*	-0.012	0.038
Weight (gm.)		1.000	-0.085	-0.111	0.209	-0.017	0.045
Temperature			1.000	0.674**	0.477**	0.064	-0.453**
S.L.'s/1000				1.000	0.518**	0.243	-0.263
% S.L.'s					1.000	0.108	-0.556**
L.L.'s/1000						1.000	0.130
% L.L.'s							1.000
Nt.'s/1000							
% Nt.'s							
Imm. R.B.C.'s/1000							
Thrombocytes							
I.N.D.							
No.Nu.							
E.N.D.							

 *Significant at the 95 per cent level   Critical Absolute Values: 5%: 0.288

**Significant at the 99 per cent level   1%: 0.372

S.L.'s/1000: Small Lymphocytes per 1000 blood cells   L.L.'s/1000: Large Lymphocytes per 1000 blood cells

% S.L.'s: Small Lymphocytes per 100 Leucocytes   % L.L.'s: Large Lymphocytes per 100 Leucocytes

Temperature = Water Temperature at Time of Autopsy

not significantly) correlated with numbers of small lymphocytes per 100 leucocytes (% S.L.'s) and significantly positively correlated with numbers of large lymphocytes per 100 leucocytes (% L.L.'s), whereas No. Nu.'s were positively correlated with % S.L.'s, and negatively correlated with % L.L.'s. Numbers of thrombocytes were negatively (but not significantly) correlated with I.N.D.'s and unrelated to No. Nu.'s. Unlike the injection experiments, lengths and weights were positively correlated with I.N.D.'s, and negatively correlated with No. Nu.'s. Numbers of immature erythrocytes per 1000 blood cells were positively correlated with I.N.D.'s and No. Nu.'s.

Nuclear diameters of epsilon cells (E.N.D.'s) were negatively correlated with S.L.'s/1000 and % S.L.'s. On the other hand, E.N.D.'s were not correlated significantly with either L.L.'s/1000 or Nt.'s/1000. However, E.N.D.'s were positively (but not significantly) correlated with % L.L.'s, and very significantly positively correlated with % Nt.'s. E.N.D.'s were correlated negatively with thrombocyte counts, although not significantly. Nuclear diameters of epsilon cells were significantly positively correlated with lengths and weights of fish, and with numbers of immature erythrocytes per 1000 blood cells.

As well as being negatively correlated with I.N.D.'s and E.N.D.'s and positively correlated with No. Nu.'s, numbers of small lymphocytes per 1000 blood cells were positively correlated with % S.L.'s and with thrombocyte counts. However, S.L.'s/1000 were not correlated significantly with either numbers of large lymphocytes or numbers of neutrophils per 1000 blood cells. No significant correlation existed between S.L.'s/1000 and either lengths or weights.

Numbers of small lymphocytes per 100 leucocytes (% S.L.'s) were negatively correlated with % L.L.'s and % Nt.'s, but were not correlated

significantly with L.L.'s/1000 or Nt.'s/1000. Although % S.L.'s and S.L.'s/1000 were very significantly positively correlated, % L.L.'s and L.L.'s/1000 were not correlated. Neither were % Nt.'s and Nt.'s/1000 significantly correlated.

The water temperature of Bertrand Creek at the time of sampling was very significantly correlated with several sample variables. Temperatures and interrenal nuclear diameters were negatively correlated, whereas temperatures and numbers of interrenal nuclei per defined area were positively correlated. Temperatures and epsilon cell nuclear diameters were also negatively correlated. A highly significant positive correlation existed between numbers of small lymphocytes per 1000 blood cells or % S.L.'s and water temperatures, whereas % L.L.'s and % Nt.'s were negatively correlated with temperatures. Temperatures and Nt.'s/1000 or L.L.'s/1000 were not significantly correlated. Thrombocyte counts were positively correlated with water temperatures. Numbers of immature erythrocytes per 1000 blood cells, on the other hand, were negatively correlated with water temperatures.

DISCUSSION

It is evident that the pituitary-interrenal axis of juvenile
coho salmon in their natural habitat undergoes a marked variation in
activity during the year of stream residence. The criteria utilized
to investigate the pituitary-interrenal axis of coho fry in the injection
experiments (differences in interrenal and epsilon cell nuclear diameters,
nucleolar prominence, interrenal cell size, hyperplasia of interrenal
tissue, frequency of mitotic figures, degree of vascularization of head
kidney) indicated functional alterations in the activity of the interrenal
tissue and epsilon cells seasonally. According to these criteria, the
pituitary-interrenal axis of juvenile coho salmon is inactive from the
time of emergence in spring, through summer and early fall, compared with
the winter sample and spring samples of yearling coho. The head kidneys
and interrenal tissue of these later stream samples displayed
histological and histometric alterations similar to those observed in coho
fry injected with mammalian ACTH, although the response was less
pronounced than that following higher dosages (0.2 or 0.4 I.U.) of ACTH.
It is noteworthy that the significant increase in mean interrenal nuclear
diameters for the winter and spring samples of yearling coho were
accompanied by significant increases in mean diameters of epsilon cell
nuclei. Furthermore, epsilon cell nuclear diameters were positively
correlated with both interrenal nuclear diameters and estimates for
interrenal cell size. These data, then, provide further support for the
hypothesis that the epsilon cells are the corticotrops in the pituitary
gland of coho salmon.

In this study, numbers of circulating small lymphocytes and thrombocytes were highest in the summer samples. The increased activity of the pituitary-interrenal axis observed in winter or spring samples of yearling coho salmon compared with summer or fall samples was accompanied by a significant lymphopenia. In addition, numbers of circulating thrombocytes were significantly decreased in the December 1968 sample compared with summer samples, although thrombocyte counts increased again in the spring samples of yearling coho, associated with rising water temperatures. Furthermore, numbers of small lymphocytes were significantly negatively correlated with estimates of interrenal cell size, nuclear diameters of interrenal cells and nuclear diameters of epsilon cells. Similar correlations between small lymphocyte counts and interrenal cell measurements were found when coho fry were injected with ACTH. Although correlation of variables does not prove a cause-effect relationship, the inter-relationships of variables measured in seasonal samples of juvenile coho are at least in accord with the hypothesis that the increased activity of the pituitary-interrenal axis participates in the seasonal variations in leucocyte counts observed.

A review of the literature reveals a limited knowledge of the effects of changing environmental conditions on blood cell counts of teleost fishes. Schlicher (1927), in an investigation of seasonal variations in blood cell counts of several species, reported that leucocyte counts in the carp, tench and goldfish were high in the summer, and decreased during the fall to a minimum in December. Low leucocyte counts were also observed in Salmo fario and Salmo irideus during winter months. Schlicher attributed this decrease in circulating leucocytes to low water temperatures, and demonstrated experimentally that a rise in

water temperature caused an increase in numbers of leucocytes in perch and goldfish. Yokoyama (1947) found a marked increase in thrombocyte counts of the perch, Perca flavescens during the summer, associated with a more rapid blood clotting time. Similarly, McKnight (1966) noted slightly lower thrombocyte counts in mountain whitefish during the months of January to March. In addition, Gardner and Yevich (1969) reported that the numbers of circulating mature thrombocytes and small lymphocytes in Fundulus heteroclitus were lowest during the winter months. Thus, these previous reports concur with the findings of this study. The present study further indicates that the season of capture must be considered in the blood cell analysis of fishes.

The strikingly low lymphocyte and thrombocyte counts of the April 1969 sample of newly-emerged coho fry are apparently inconsistent with the hypothesis that a lymphopenia and thrombocytopenia reflect increased pituitary-interrenal activity, since the interrenal tissue of this sample was relatively inactive according to the criteria for activity employed in this study. However, Katz (1949), while investigating the hematology of this same species during different stages of development, noted that the blood of alevins had low erythrocyte counts and was almost devoid of leucocytes. Furthermore, the blood of newly-emerged coho fry was still nearly devoid of leucocytes, while the erythrocytes, although more numerous than before, were approximately 20% immature cells. Thus, the leucopenia of newly-emerged coho reported in the present study is probably a normal developmental phenomenon, and should not be considered as a discrepancy in the hypothesis.

Thrombocyte counts of juvenile coho are influenced by the activity of the pituitary-interrenal axis, as shown by the thrombocytopenia observed

following ACTH, dexamethasone or cortisol injections. In this study, numbers of thrombocytes were negatively correlated with nuclear diameters of epsilon and interrenal cells, although these correlations were not statistically significant. On the other hand, thrombocyte counts were positively correlated with water temperatures. Therefore, it appears that the thrombocytes are more directly influenced by water temperature than by activity of the pituitary-interrenal axis. However, this supposition is far from substantiated, and requires further investigation.

Adrenocortical hormones are known to influence mammalian intermediary metabolism, stimulating protein catabolism in extrahepatic tissues, particularly skeletal muscle, and promoting gluconeogenesis, with a resulting increase in blood glucose and liver glycogen levels (Long et al., 1940; Cannon et al., 1956; Bellamy and Leonard, 1964). Evidence for the metabolic role of corticosteroids in fishes, although still scarce, is gradually emerging. Except for minor modifications, corticosteroids have a similar action in both teleost fishes and in mammals (Black et al., 1961; Storer, 1967; Chester Jones et al., 1969). In various teleosts, an increase in blood glucose or liver glycogen levels follows administration of ACTH or corticosteroids (Nace, 1955; Falkmer, 1961; Robertson et al., 1963; Kumar et al., 1966; Oguri and Nace, 1966; Butler, 1968). Butler (1968) reported that hypophysectomy or administration of a metabolic inhibitor of corticosteroid synthesis to Anguilla rostrata caused a significant decrease in liver glycogen levels. Storer (1967) found that administration of cortisol to intact goldfish produced a decrease in body weight, and an increase in ammonia excretion and liver GPT activity. These findings demonstrate that cortisol-type steroids in teleosts, as in mammals, promote gluconeogenesis.

It is well established that the plasma corticosteroid levels of salmonids are markedly elevated during their spawning migration (Hane and Robertson, 1959; Robertson et al., 1961; Schmidt and Idler, 1962), and are accompanied by an obvious hypertrophy and hyperplasia of the interrenal tissue (Robertson and Wexler, 1959). These changes are associated with a substantial catabolism of parietal muscle protein (Idler and Clemens, 1959; Robertson et al., 1961), marked depletion of lymphocytes from the spleen and thymus (Robertson and Wexler, 1960; Robertson et al., 1961), hyperglycemia (Robertson et al., 1961) and an elevation of liver glycogen (Chang and Idler, 1960). Similar changes were observed when immature rainbow trout were administered cortisol acetate pellets (Robertson et al., 1963). Thus, this increase of adrenocortical activity in salmonids during their spawning migration, coupled with a substantial conversion of muscle protein to carbohydrate, provides fasting fish with energy for normal metabolism, for the development of gonads and for increased muscular activity. It seems likely, therefore, that in the salmonids, as in other teleosts, glucocorticoids are potentially available to stimulate utilization of muscle protein for the provision of metabolic energy.

In the present investigation, ambient water temperatures at the time of autopsy of juvenile coho salmon were very significantly negatively correlated with nuclear diameters of interrenal cells and epsilon cells and with estimates of interrenal cell size, and were very significantly positively correlated with thrombocyte counts and small lymphocyte counts. Furthermore, according to the other histological criteria used in this study for assessing interrenal activity, the interrenal tissue of the December 1968 sample (water temp. 4°C.) was far more active than that of preceding samples from warmer waters, and was more active than the

interrenal tissue of the April 1968 or March 1969 samples of yearling coho (water temperatures 8.5°C. and 10°C. respectively). Therefore, if these criteria are accepted as indicative of interrenal activity, the activity of the pituitary-interrenal axis of juvenile coho salmon sampled seasonally from Bertrand Creek is inversely related to ambient water temperatures. Further evidence for an inverse relationship between water temperatures and increased activity of the interrenal tissue of coho salmon is provided in Section III.

Although no previous reports have appeared on the activity of the pituitary-interrenal axis of teleost fishes in relation to variations in ambient water temperatures, some circumstantial evidence is available which does imply a relationship. Several accounts of lymphopenia or thrombocytopenia in winter samples of teleosts have been previously cited. Schuh and Nace (1961) reported a seasonal increase in blood glucose levels in toadfish as water temperatures declined in winter. Furthermore, fish kept at low temperatures in early summer showed a hyperglycemia appropriate to the season of corresponding low environmental temperatures (Nace and Schuh, 1961).

During temperature acclimation, the metabolism of the organism is fundamentally reorganized (Hochachka, 1967). It is interesting that Fry and Hochachka (1970) have proposed a model attempting to account for the metabolic adjustments observed in teleost fishes during cold acclimation that requires active participation of both glucocorticoids and insulin. This model is based on the observation that, in tissue of cold acclimated fish, compared with tissue of warm acclimated ones, the glycogen synthesis rate is increased, glycolysis rate is increased up to five-fold, lipogenesis is activated, and the synthesis of proteins and nucleic acids in the liver

is increased. According to the model, glucocorticoids release amino acids from the peripheral tissues into the general circulation. These amino acids activate pre-existing gluconeogenic enzymes in the liver. Secondly, glucocorticoids induce _de novo_ synthesis of key gluconeogenic enzymes (thought to be isozymic variants of those present in warm acclimation). The resulting increased glucose production causes the release of insulin from pancreatic beta cells. Insulin slows up gluconeogenic flux, activates certain enzymes that channel glucose into glycolysis and glycogen, and activates malic enzyme (which generates TPNH for fat synthesis). Thus this model proposes that the adjustments seen in fish liver during cold acclimation are those which would be predicted on the assumption that gluconeogenesis is activated by glucocorticoids, and glycolysis and lipogenesis by insulin. A marked hypertrophy and hyperplasia of the pancreatic beta cells of spawning Pacific salmon or steelhead trout have been previously reported (Robertson and Wexler, 1960; Robertson _et al._, 1961). Therefore, pancreatic beta cell stimulation observed in these studies and postulated in the model of Fry and Hochachka (1970) may be a normal consequence of adrenocorticoid-induced hyperglycemia in teleost fishes.

It is tempting to suggest that the increased activity of the pituitary-interrenal axis observed in the winter sample of juvenile coho is the result of cold acclimation. However, other seasonal environmental alterations could conceivably account for this stimulation. The availability of food supply, interactions of fish, alterations in stream flow and photoperiod must all be considered as alternative hypotheses. Examination of gut contents on autopsy revealed no decrease in amount of food consumed by the winter sample of coho fry compared with previous

samples; however, the increased effort required to obtain adequate food supply is unknown. Stream velocity and turbidity during capture of the winter sample were far less than observed when the autumn sample was obtained. No critical observations of behavior of juvenile coho were made in this study, although Chapman (1962) and Hartman (1965) reported very low levels of aggressiveness of coho fry in streams during the winter. Although seasonal variation in photoperiod is known to be involved in several endocrinological responses of teleost fishes (Hoar, 1965), no studies have investigated the interrenal response to alterations in photoperiod. Therefore, speculation as to the involvement of photoperiod in this study awaits experimental manipulation. The effect of an altered photoperiod on the pituitary-interrenal axis of juvenile coho salmon is considered in Section III.

The interrenal tissue of the March 1969 and April 12, 1968 samples, although still hyperplastic, was variable in appearance, displaying considerable signs of atrophy. Interrenal tissue of the April 26, 1969 sample, on the other hand, appeared very active, with no atrophy evident. These samples of fish also differed in appearance. In the earlier spring samples with somewhat atrophic interrenals, parr marks were still evident, whereas these were obscured owing to increased silvering in the April 1969 sample. This silvering, due to guanine and hypoxanthine deposition in the skin and scales (Johnston and Eales, 1967), typifies the parr-smolt transformation of certain species of salmon, including the coho.

The parr-smolt transformation of young salmon prior to seaward migration is accompanied by numerous biochemical, histological, physiological and behavioral changes (Baggerman, 1960a,b; Barrington, 1961; Hoar, 1965; Conte et al., 1966; Vanstone and Markert, 1968). The

biochemical changes are profound, probably involving many enzyme changes, and the nature of the nucleotides and body lipids (Hoar, 1965; Vanstone and Markert, 1968). The endocrine system has also been implicated in the regulation of these changes. The thyroid gland is more active in smolts of the Atlantic salmon (Hoar, 1939) and of several species of the Pacific salmon (Hoar and Bell, 1950; Eales, 1963) than in parr of these species. The increased activity of the interrenal tissue during the parr-smolt transformation observed in this investigation has been previously reported for the Atlantic salmon (Salmo salar). Fontaine and Olivereau (1957) observed hypertrophy of nuclei and nucleoli and increased mitotic activity of the interrenal cells of the Atlantic salmon smolts compared with parr. Furthermore, the concentration of corticosteroids was much higher in the plasma of Atlantic salmon smolts than in parr (Fontaine and Hatey, 1954). Therefore, the data reported here, as well as those available in the literature, provide support for the hypothesis that the pituitary-interrenal axis of certain salmonids is activated during the parr-smolt transformation, and that this activation is necessary to provide increased metabolic energy for the numerous histological, biochemical and physiological alterations associated with this transformation. It would be worth-while to determine whether or not hypophysectomized fish, or fish treated with a metabolic inhibitor of cortisol synthesis such as metopirone, could undergo this transformation from parr to smolt.

SECTION III: VARIATIONS IN INTERRENAL ACTIVITY AND RELATED HEMATOLOGICAL
CHANGES IN JUVENILE COHO SALMON EXPOSED TO DISSIMILAR
ENVIRONMENTS WITHIN THE LABORATORY

INTRODUCTION

In Section II, seasonal variations in the activity of the pituitary-
interrenal axis of juvenile coho salmon were reported. The increased
activity of this axis in the winter sample of coho fry was tentatively
attributed to the marked decrease in water temperature. However, the
changing length of daily photoperiod is responsible for seasonal cycles in
activity of several endocrine organs of fishes (Hoar, 1958, 1965). Since
seasonally changing photoperiods also might influence the activity of the
pituitary-interrenal axis of fish, it was decided to study the effect of
changes in this variable within the laboratory on the interrenal activity
of juvenile coho salmon. Therefore, this section reports on the effects of
varying periods of continuous darkness or continuous light on the interrenal
activity of juvenile coho salmon. The response of the interrenal tissue of
fish exposed to varying periods of a continuously flashing light also is
investigated in order to ascertain if (or to what extent) an atypical
environment stimulates the pituitary-interrenal axis, and if this
activation is perpetuated while the altered environment is maintained. In
addition, the relationship of the numbers of circulating leucocytes to the
activity of the interrenal tissue in each of these experiments is discussed.
Variations in ambient water temperatures in these experiments are correlated
with alterations in leucocyte counts and histometric estimations of interrenal
cell activity to determine if, in fact, these variables are affected by
environmental temperature. Finally, the interrelationships between

variables measured in all the laboratory experiments and from seasonal stream samples are described and discussed.

MATERIAL AND METHODS

Continuous-Darkness Experiments

Approximately three hundred coho fry were captured from Bertrand

Creek between November 4th and November 17th, 1967 by pole seine, and taken

in buckets to the laboratory, where they were placed in holding tanks A and

B (Material & Methods Section I), 150 fish per tank. All fish were

initially maintained under a natural photoperiod: this was followed by

adjustment of the photoperiod by small daily increments, until by December

29, 1967 fish were receiving a twelve hour light - twelve hour dark photo-

period. After this period of acclimation, experiments in continuous darkness

were commenced.

The procedure for each experiment involving continuous darkness was

the same. Twelve fish were removed from the stock tanks (six per tank) by

netting, and one fish was placed in each of the experimental compartments.

Compartments were illuminated with the same twelve hour photoperiod as

holding tanks. These compartments are described in Material & Methods Section

I. However, neither compartments nor holding tanks were provided with gravel

bottoms or artificial cover (Fig. 5). Also, lids of compartments were

equipped with black flexible tubing. Since brineshrimp could be passed down

this tubing without opening the lids, fish could be fed without exposure to

light.

Following transfer of fish to identical experimental compartments,

lids were sealed with opaque tape to ensure light-tight conditions. Each

fish was fed brineshrimp daily through the flexible tubing until termination

of the experiment. In each experiment, all twelve fish were maintained in

identical environmental conditions for 7 days. Following this, the power

source to six compartments was disconnected during the dark cycle of the photoperiod. Fish in these compartments were considered as experimental, while those in compartments with functional lights were controls. Thus the control fish continued to receive a twelve hour photoperiod, while the experimental fish were maintained in complete darkness for a certain number of days in each experiment. Five separate experiments were performed, each involving a different length of darkness for the experimental group of six fish. Fish were exposed to 4, 8, 12, 16 or 20 days of total darkness, while the control group of six fish in each case received a twelve hour photoperiod.

To terminate each experiment, fish were removed from compartments in two manners: anesthetizing with 0.005% MS222 (utilizing feeding tubes in lids) followed by rapid removal and dissection, or simply by netting quickly. All twelve fish were sacrificed during the dark phase of the photoperiod, so that both control and experimental fish would receive the same unavoidable exposure to light immediately prior to sacrifice. Six fish were sampled by netting from each of holding tanks A and B at the time of termination of the final continuous darkness experiment.

The autopsy procedure and histological techniques utilized are described in Section I. Techniques of karyometry and statistical evaluation of results are also given in Section I. For each experimental treatment, mean interrenal nuclear diameters, numbers of interrenal nuclei per defined area of interrenal tissue and mean blood cell counts of fish sacrificed with or without anesthesia were determined and compared.

Continuous-Light Experiments

On November 9, 1968, approximately one hundred coho fry were captured from Bertrand Creek by pole seine, and transported to the laboratory, where they were placed in holding tank C. Fish were initially maintained

under a natural photoperiod, followed by adjustment by small increments daily, until by January 10, 1969 fish were receiving a twelve hour light - twelve hour dark photoperiod. All fish were fed frozen brineshrimp daily throughout the experiments.

Continuous-light experiments were commenced on January 27, 1969. Twelve fish were removed from the holding tank by netting, and placed in individual experimental compartments. Each compartment was illuminated with the same twelve hour photoperiod as holding tanks. Neither compartments nor holding tanks were provided with gravel bottoms or artificial cover. control samples of four fish each were obtained after 1, 2 and 4 days residence in compartments.

In each continuous-light experiment, all fish transferred to compartments were exposed to a twelve hour photoperiod for seven days. After this period of acclimatization the power supply to half of the compartments was fixed to a permanent source, while conditions for the remaining compartments were unaltered. Therefore, the control fish continued to receive a twelve hour photoperiod, while the experimental fish were exposed to continuous illumination for a certain number of days in each experiment. Samples of four fish each received continuous illumination for periods of 1, 2, 4, 8 and 14 days, while the control group of four fish in each experiment were exposed to a twelve hour photoperiod for the same number of days. Stock samples were obtained from the holding tank at the beginning and termination of this series of experiments. A further stock sample was obtained during these experiments.

To terminate each experiment, all fish were netted and sacrificed quickly, without prior anesthetizing. The procedures for autopsy, histological techniques utilized, karyometric techniques and statistical evaluation of

results are described in Material & Methods of Section I.

Flashing-Light Experiments

Approximately one hundred coho fry were captured from Bertrand Creek by pole seine on November 16, 1968. These fish were transported to the laboratory, where they were placed in a large fibre-glass stock tank (described in Section I), and maintained under a natural photoperiod. All fish were fed brineshrimp daily throughout these experiments until sacrificed.

On March 10, 1969, thirty-five fish were transferred to each of holding tanks A and B in preparation for the flashing-light experiments. These fish received a twelve hour photoperiod. Neither holding tanks nor experimental compartments were provided with a gravel bottom or artificial cover. Following a four week period of acclimatization to holding tanks, flashing-light experiments were commenced.

On April 7, 1969, twelve fish (six from each holding tank) were netted and transferred to individual experimental compartments (described in Section I). These compartments were regulated with the same twelve hour photoperiod as holding tanks. Fish were sampled from these compartments in the usual manner after 1, 2 and 4 days residence. In each experiment involving continuously flashing lights, all fish transferred to individual compartments were exposed to a twelve hour light - twelve hour dark photoperiod for seven days. Following this acclimatization period, the power supply to half of the compartments was relayed through a Noma A.C. Flasher (Cat. #558), which caused the lights within these compartments to flash on and off every two to five seconds continuously. Hence the control fish continued to receive a twelve hour photoperiod, while the experimental fish were exposed to a continuously flashing light for a certain number of days in

each experiment. Samples of four fish each were exposed to a flashing light for periods of 12 hours, 1, 2, 4, 8 and 16 days, while the control group of four fish in each experiment received a twelve hour photoperiod for the same number of days. Holding tanks were sampled at the beginning of and also during this series of experiments.

To terminate experiments, each fish was netted and sacrificed quickly, without prior anesthetizing. Procedures for autopsy, histological techniques utilized, karyometric techniques and statistical evaluation of results are presented in Section I.

Correlation Matrix of Variables Measured from all Experiments

Correlation coefficients relating variables measured in fish involved in all experiments (including seasonal samples from Bertrand Creek) were determined as described in Section I of Material and Methods. However, variables measured in fish receiving injections (saline, ACTH, cortisol acetate or dexamethasone) were not included.

RESULTS

A. CONTINUOUS-DARKNESS EXPERIMENTS

Histology of the Head Kidney and Interrenal Gland

Head kidneys of the stock-tank sample of fish were moderately vascularized. Interrenal tissue was moderately to very hyperplastic. Interrenal cells within many clusters appeared somewhat atrophic, with irregularly shaped nuclei and unevenly stained, vacuolated cytoplasm. No mitotic figures were observed within the interrenal tissue.

Head kidneys of fish maintained in total continuous darkness for 4 days were extremely well vascularized. Interrenal tissue for all fish was markedly hyperplastic. Interrenal cells were very hypertrophied, with enlarged nuclei and prominent nucleoli. Cytoplasm of these cells was evenly stained and non-vacuolated. A few clusters of somewhat atrophic interrenal cells were observed also. Mitotic figures were observed occasionally. Head kidneys and interrenal tissue of corresponding control fish were similar in appearance to those of experimental fish.

The interrenal tissue of fry maintained in darkness for 8 days was extremely hyperplastic. Cells were markedly hypertrophied, with enlarged nuclei and prominent nucleoli. Cytoplasm was evenly stained and non-vacuolated. Mitotic figures were infrequently observed. No atrophic cells were seen. Head kidneys were very well vacularized. Head kidneys and interrenal tissue of corresponding control fish were similar to those of the 8 day darkness fish.

Extensive hyperplasia of interrenal tissue occurred within the head kidneys of fish exposed to total continuous darkness for 12 days. However, many clusters of interrenal cells appeared atrophic. Interrenal tissue of

control fish for this experiment was similar, although somewhat more atrophic. Mitotic figures were observed very infrequently within the interrenal tissue of fish exposed to darkness, but were not observed in interrenal tissue of control fish.

Head kidneys of fish maintained in darkness for 16 days were not as well vascularized as were those of previous samples. Very extensive hyperplasia of interrenal tissue was observed. However, considerable atrophy of interrenal cells was present, although a few clusters of cells appeared to be stimulated. Occasional mitotic figures were seen. Interrenal tissue of controls also exhibited extensive hyperplasia. Interrenal cells were even more atrophic than those of fish exposed to 16 days of darkness. Nuclei were irregularly shaped and closely apposed to one another, with inconspicuous nucleoli. Cytoplasm was unevenly stained and vacuolated. Mitotic figures were not observed.

Head kidneys from both controls and fish exposed to continuous darkness for 20 days were moderately to well vascularized. In both samples, interrenal tissue was moderately to extensively hyperplastic. However, extensive atrophy of the interrenal cells was evident, similar to the previous control sample. No differences were observed between controls and fish maintained in darkness for 20 days.

The mean diameters of the interrenal cell nuclei for all samples of fish involved in the continuous darkness experiments are presented in Figure 57 and Table XII. Using Scheffe's test for multiple comparisons, mean interrenal nuclear diameters (I.N.D.'s) for fish exposed to continuous darkness for 4 days and corresponding controls were very significantly larger than the mean nuclear diameter of fish sampled from the holding tanks at the same time. Mean I.N.D.'s of 4 day darkness and control fish were also

TABLE XII

MEAN INTERRENAL NUCLEAR DIAMETERS (I.N.D.), NUMBER OF INTERRENAL NUCLEI PER DEFINED AREA OF INTERRENAL TISSUE (NO.NU.), AND EPSILON CELL NUCLEAR DIAMETERS (E.N.D.) OF JUVENILE COHO SALMON INVOLVED IN THE CONTINUOUS-DARKNESS EXPERIMENTS

Number of Fish	Sample	Water Temp. (°C.)	I.N.D. (microns) Mean ± S.E.	NO.NU. Mean ± S.E.	E.N.D. (microns) Mean ± S.E.
12	May 15/68 Stock	9.5	6.42 ± 0.01	24.0 ± 0.7	5.32 ± 0.03 *($\overline{6}$)
6	May 15/68 Dark 4 Days	9.5	6.75 ± 0.02	21.2 ± 1.1	5.51 ± 0.03 *($\overline{6}$)
6	May 15/68 11 Day Control	9.5	6.69 ± 0.02	22.6 ± 0.4	5.47 ± 0.03 *($\overline{6}$)
6	February 3/68 Dark 8 Days	5.0	6.56 ± 0.02	-	-
6	February 3/68 15 Day Control	5.0	6.50 ± 0.02	-	-
6	April 29/68 Dark 12 Days	9.0	6.53 ± 0.02	23.4 ± 1.2	-
6	April 29/68 19 Day Control	9.0	6.42 ± 0.02	24.9 ± 0.6	-
6	April 5/68 Dark 16 Days	8.0	6.44 ± 0.02	24.8 ± 0.6	-
6	April 5/68 23 Day Control	8.0	6.44 ± 0.02	26.0 ± 0.7	-
6	March 7/68 Dark 20 Days	12.5	6.34 ± 0.02	27.0 ± 1.1	-
6	March 7/68 27 Day Control	12.5	6.41 ± 0.02	27.0 ± 1.1	-

*Sample size for Epsilon cell nuclear diameters

FIGURE 57:   Effect of continuous darkness on nuclear diameters of
             interrenal cells, and on small-lymphocyte, thrombocyte
             and neutrophil counts in juvenile coho salmon.

116
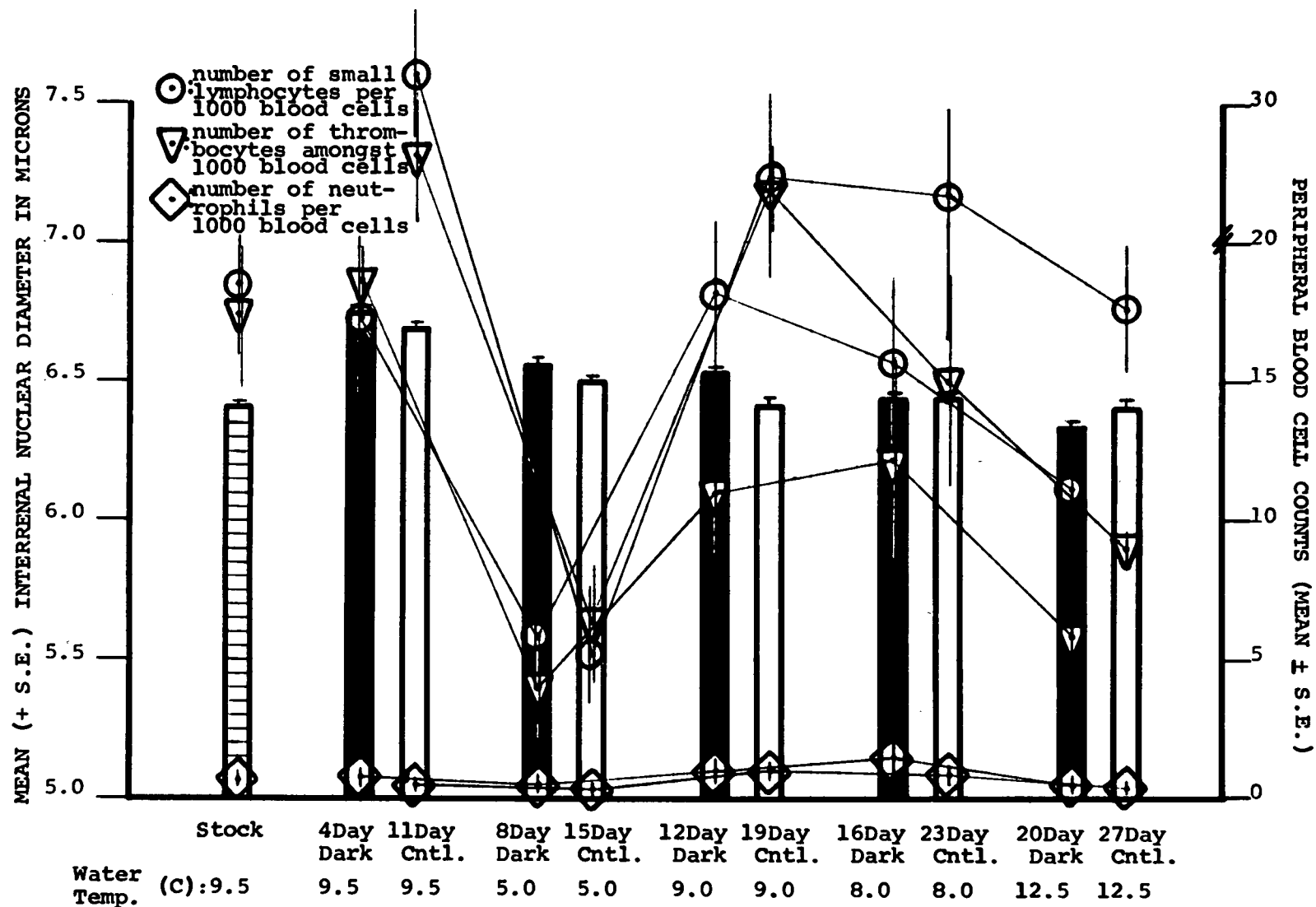
FIGURE 58:   Interrenal cell size* in juvenile coho salmon from

the continuous-darkness experiments.

MEAN (+ S.E.) NUMBER OF INTERRENAL
CELL NUCLEI PER DEFINED AREA**
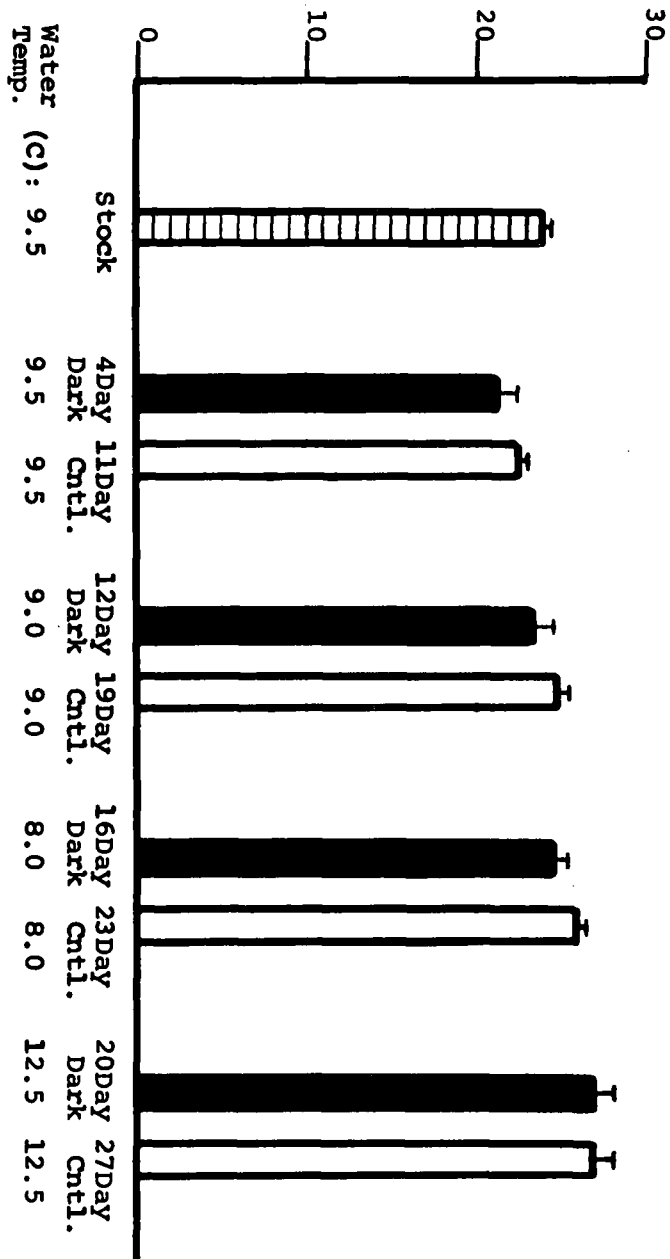
Water
Temp. (C):

Stock   9.5

4Day   9.5
Dark

11Day   9.5
Cntl.

12Day   9.0
Dark

19Day   9.0
Cntl.

16Day   8.0
Dark

23Day   8.0
Cntl.

20Day   12.5
Dark

27Day   12.5
Cntl.

*Interrenal cell size is inversely related to the
 number of interrenal cell nuclei per defined area.

**Defined area is 450 μ² of interrenal tissue.

significantly larger than means for darkness or control fish in all other experiments. In each experiment, the mean I.N.D. of fish exposed to continuous darkness did not differ significantly from that of corresponding controls. However, mean I.N.D.'s of darkness-maintained fish were larger than respective controls in the 4, 8 and 12 day experiments. Also, the mean diameters of interrenal cell nuclei decreased consistently with increasing periods of exposure of fish to continuous darkness.

The mean interrenal cell size for each sample is represented in Figure 58 and Table XII. Unfortunately, interrenal cell size was not determined for fish involved in the 8 day-darkness experiment. Samples of fish exposed to total darkness for four days and corresponding controls both exhibited a decrease in mean numbers of interrenal nuclei per defined area of interrenal tissue (increase in cell size) when compared with the stock sample. However, this decrease was not significant according to Scheffe's test. Mean numbers of interrenal cell nuclei per defined area (No. Nu.'s) consistently increased for both dark-maintained fish and corresponding controls with increasing periods of holding in compartments prior to sacrifice. No. Nu.'s for the darkness-maintained fish and corresponding controls in the 20 day darkness experiment were significantly larger than corresponding samples from the 4 day-darkness experiment. Furthermore, mean interrenal cell size of fish maintained in continuous darkness was larger (smaller No. Nu.'s) than values for corresponding controls in each of the 4, 12 and 16 day experiments, although means did not differ significantly.

Mean nuclear diameters of epsilon cells in the rostral pars distalis were determined for the stock-tank sample, and for fish exposed to continuous darkness for 4 days and for the corresponding controls. Values are presented in Table XII. Mean epsilon cell nuclear diameters for both 4 day-

darkness fish and controls were very significantly (p < 0.01) larger than the mean nuclear diameter of the stock sample. However, mean nuclear diameters for the 4 day-darkness sample and corresponding control sample did not differ significantly.

Mean Blood Cell Counts

The mean blood cell counts for samples of juvenile coho involved in the continuous-darkness experiments are presented in Table XIII. Mean numbers of small lymphocytes per thousand blood cells (S.L.'s/1000) for fish exposed to continuous total darkness were smaller than mean values for corresponding controls in each of the 4, 12, 16 and 20 day-darkness experiments (Fig. 57 and Table XIII). However, according to Student's t test, these differences were not significant (p ⩾ 0.05). In the 8 day-darkness experiment (water temp. 5°C.), S.L.'s/1000 for both fish exposed to continuous darkness and control fish were significantly lower than values for all other samples.

Mean numbers of small lymphocytes per hundred leucocytes (% S.L.'s) were lowest for both controls and experimental fish in the 8 day-darkness experiment compared with values for all other samples. The mean percentage of small lymphocytes for controls from the 8 day-darkness experiment was very significantly smaller than means for controls from the 4 or 12 day-darkness experiments. In all experiments, % S.L.'s did not differ significantly between fish exposed to continuous darkness and corresponding controls, although mean of fish maintained in darkness for 4, 12 or 16 days were lower than controls.

Mean numbers of large lymphocytes per thousand blood cells were negligible for all samples. The mean numbers of large lymphocytes per

TABLE XIII

BLOOD CELL COUNTS OF JUVENILE COHO SALMON INVOLVED IN THE
CONTINUOUS-DARKNESS EXPERIMENTS

No. of Fish	Treatment/ gm.wt./day for 7 Days	Water Temp. (°C.)	Mat. R.B.C.'s/ 1000	Imm. R.B.C.'s/ 1000	S.L.'s/ 1000	% S.L.'s	L.L.'s/ 1000	% L.L.'s	Nt.'s/ 1000	% Nt.'s	Throm.	Hct.
						MEAN ± S.E.						
6	Stock	9.5	922.8 (7.4)	57.8 (7.2)	18.5 (2.6)	94.9 (1.1)	0.2 (0.1)	0.7 (0.2)	0.7 (0.2)	4.3 (1.0)	17.4 (2.7)	31.5 (0.6)
6	Dark 4 Days	9.5	951.0 (12.1)	30.0 (10.8)	17.3 (3.2)	85.3 (4.0)	0.8 (0.5)	3.5 (1.5)	0.8 (0.4)	9.8 (2.6)	18.7 (1.3)	34.0 (2.1)
6	11 Day Control	9.5	938.7 (6.0)	28.5 (6.7)	32.0 (4.7)	95.5 (1.2)	0.3 (0.2)	0.5 (0.3)	0.5 (0.2)	2.5 (0.3)	26.2 (4.8)	32.8 (2.9)
6	Dark 8 Days	5.0	924.8 (7.6)	68.8 (7.7)	5.8 (1.9)	73.2 (4.1)	0.0	4.7 (0.7)	0.5 (0.3)	20.8 (3.0)	4.0 (1.9)	-
6	15 Day Control	5.0	962.5 (10.3)	32.0 (9.9)	5.2 (1.8)	73.2 (5.4)	0.0	6.7 (1.8)	0.3 (0.2)	17.8 (4.1)	6.3 (2.1)	-
6	Dark 12 Days	9.0	928.5 (4.9)	52.0 (7.4)	18.2 (3.4)	86.7 (4.5)	0.0	1.5 (0.7)	1.0 (0.5)	11.2 (3.5)	11.0 (2.4)	32.6 (2.3)
6	19 Day Control	9.0	888.8 (7.8)	85.2 (7.4)	24.7 (6.1)	95.8 (0.9)	0.3 (0.2)	0.8 (0.4)	1.0 (0.3)	3.0 (0.6)	23.8 (3.2)	25.1 (2.5)
6	Dark 16 Days	8.0	958.0 (8.7)	24.5 (6.6)	15.7 (3.0)	84.3 (4.7)	0.3 (0.2)	2.7 (1.2)	1.5 (0.8)	11.5 (3.8)	12.2 (3.6)	29.8 (2.1)
6	23 Day Control	8.0	933.2 (12.2)	42.6 (7.9)	23.4 (6.9)	92.8 (2.2)	0.0	1.4 (0.2)	0.8 (0.4)	5.4 (2.3)	15.0 (3.8)	30.1 (2.6)
6	Dark 20 Days	12.5	944.7 (9.4)	43.0 (9.0)	11.2 (1.6)	92.2 (0.7)	0.7 (0.3)	1.3 (0.7)	0.5 (0.3)	5.7 (0.3)	5.8 (1.1)	38.6 (4.6)
6	27 Day Control	12.5	910.6 (12.0)	70.4 (11.8)	17.6 (2.3)	90.0 (1.5)	0.4 (0.2)	3.2 (1.1)	0.4 (0.2)	5.6 (1.1)	9.0 (0.6)	38.4 (2.9)

hundred leucocytes (% L.L.'s) were largest for both experimental fish and controls in the 8 day-darkness experiment compared with other samples.

The mean numbers of neutrophils per thousand blood cells (Nt.'s/ 1000) were small for all samples (Table XIII and Fig. 57), and did not differ significantly in any comparisons. Mean numbers of neutrophils per hundred leucocytes (% Nt.'s) were highest for both experimental and control samples in the 8 day-darkness experiment compared with all other samples. The mean percentage of neutrophils for controls from the 8 day-darkness experiment was significantly larger than means for controls from the 4 or 12 day-darkness experiments.

Numbers of immature erythrocytes varied greatly from fish to fish in all samples, as evidenced by the high S.E.'s. No significant differences were found when mean immature erythrocyte counts for different samples were compared. Mean hematocrit values for each sample also did not differ significantly.

Mean numbers of thrombocytes amongst one thousand blood cells for fish maintained in continuous darkness were smaller than mean values for corresponding controls in each of the 4, 8, 12, 16 and 20 day-darkness experiments (Fig. 57 and Table XIII). According to Student's t test, these differences were significant (p < 0.05) for the 4, 12, and 20 day-darkness experiments, but not for the 8 or 16 day-darkness experiments. Thrombocyte counts were lower for both experimental and control samples in the 8 and 20 day-darkness experiments compared with the 4, 12 or 16 day-darkness experiments. In the 8 day-darkness experiment, the mean thrombocyte count for controls was very significantly smaller than mean values for controls from the 4 or 12 day-darkness experiments. The mean count for fish maintained in darkness for 8 days was significantly lower than the mean count for fish maintained

in darkness for only 4 days. Water temperature at time of sacrifice was 5°C. for the 8 day experiment, and 12.5°C. for the 20 day experiment. However, fish involved in the 20 day-darkness experiment had been maintained in 5°C. water for a considerable length of time up until two weeks prior to sacrifice. At this time, water temperatures climbed sharply to 12.5°C. due to a malfunctioning water supply system.

In order to ascertain whether or not the method of capture prior to sacrifice affected the variables measured, mean interrenal nuclear diameters, numbers of interrenal nuclei per defined area and mean blood cell counts of fish sacrificed with or without prior anesthesia were determined and compared for each experimental treatment. No consistent differences due to method of capture were found in mean blood cell counts, interrenal nuclear diameters or numbers of interrenal nuclei per defined area of interrenal tissue when values for anesthetized or non-anesthetized fish from each sample were compared.

Correlation Coefficients of Variables Measured in the Continuous-Darkness Experiments

A correlation matrix for all variables measured in juvenile coho salmon involved in the continuous-darkness experiments is presented in Table XIV. Summary statistics for each variable are presented in Table V of Appendix II.

Interrenal nuclear diameters (I.N.D.'s) and numbers of interrenal nuclei per defined area of interrenal tissue (No. Nu.'s) were negatively correlated. Neither I.N.D.'s nor No. Nu.'s were correlated significantly with numbers of small lymphocytes, large lymphocytes or neutrophils per thousand blood cells. Furthermore, diameters of interrenal cell nuclei were not correlated with the numbers of small lymphocytes, large

TABLE XIV

CORRELATION MATRIX OF VARIABLES MEASURED FROM JUVENILE COHO SALMON
INVOLVED IN THE CONTINUOUS-DARKNESS EXPERIMENTS

	Nt.'s/1000	% Nt.'s	Imm. R.B.C.'s/ 1000	THROM.	I.N.D.	**No.Nu.
Length (cm.)	0.228	-0.390**	0.029	0.441**	0.264*	-0.270
Weight (gm.)	0.206	-0.380**	0.011	0.433**	0.209	-0.242
Hematocrit	-0.283*	0.123	-0.049	-0.416**	-0.044	-0.023
Temperature	0.046	-0.486**	0.193	0.119	0.072	0.259
S.L.'s/1000	0.238*	-0.477**	0.004	0.700**	0.180	-0.111
% S.L.'s	0.059	-0.962**	0.275*	0.429**	0.088	0.206
L.L.'s/1000	0.160	0.085	-0.237	-0.100	-0.098	-0.128
% L.L.'s	-0.056	0.656**	-0.218	-0.277*	-0.004	-0.137
Nt.'s/1000	1.000	-0.033	-0.085	0.216	-0.001	-0.036
% Nt.'s		1.000	-0.259*	-0.447**	-0.117	-0.155
Imm. R.B.C.'s/1000			1.000	0.055	-0.183	0.283*
Thrombocytes				1.000	0.227	-0.095
I.N.D.					1.000	-0.440**
No.Nu.						1.000

*Significant at the 95 per cent level
**Significant at the 99 per cent level

Critical Absolute Values: 5%: 0.250
                            1%: 0.325

Nt.'s/1000:   Neutrophils per 1000 blood cells
% Nt.'s: Neutrophils per 100 Leucocytes
Imm. R.B.C.'s/
     1000:   Immature Erythrocytes per 1000 blood cells
THROM.:   Thrombocytes amongst 1000 blood cells

I.N.D.:   Interrenal Nuclear Diameter
No.Nu.:   Number of Interrenal Nuclei per
          Defined Area of Interrenal Tissue

**   Critical Absolute Values for No.Nu.'s: 5%: 0.273
**   1%: 0.354

TABLE XIV

CORRELATION MATRIX OF VARIABLES MEASURED FROM JUVENILE COHO SALMON
INVOLVED IN THE CONTINUOUS-DARKNESS EXPERIMENTS

	LENGTH	WEIGHT	HEMATOCRIT	TEMPERATURE	S.L.'s/1000	% S.L.'s	L.L.'s/1000	% L.L.'s
Length (cm.)	1.000	0.966**	0.043	0.228	0.451**	0.454**	0.019	-0.433**
Weight (gm.)		1.000	0.092	0.254*	0.477**	0.441**	0.020	-0.443**
Hematocrit			1.000	0.479**	-0.307*	-0.162	0.187	0.192
Temperature				1.000	0.221	0.491**	0.324*	-0.396**
S.L.'s/1000					1.000	0.492**	-0.099	-0.365**
% S.L.'s						1.000	-0.108	-0.809**
L.L.'s/1000							1.000	0.178
% L.L.'s								1.000
Nt.'s/1000								
% Nt.'s								
Imm. R.B.C.'s/1000								
Thrombocytes								
I.N.D.								
No.Nu.								
E.N.D.								

*Significant at the 95 per cent level   Critical Absolute Values: 5%: 0.250

**Significant at the 99 per cent level   1%: 0.325

S.L.'s/1000: Small Lymphocytes per 1000 blood cells   L.L.'s/1000: Large Lymphocytes per 1000 blood cells

% S.L.'s: Small Lymphocytes per 100 Leucocytes   % L.L.'s: Large Lymphocytes per 100 Leucocytes

Temperature = Water Temperature at time of Autopsy

lymphocytes or neutrophils per hundred leucocytes. Thrombocyte counts were not correlated with either interrenal nuclear diameters or numbers of interrenal nuclei per defined area.

Numbers of small lymphocytes per thousand blood cells (S.L.'s/1000) were positively correlated with both numbers of small lymphocytes per hundred leucocytes and thrombocyte counts. Thrombocyte counts were also positively correlated with the percentage of small lymphocytes. S.L.'s/1000 were not correlated with numbers of large lymphocytes per thousand blood cells, but were significantly positively correlated with numbers of neutrophils per thousand blood cells. Neither large lymphocytes per thousand cells and large lymphocytes per hundred leucocytes nor neutrophils per thousand cells and neutrophils per hundred leucocytes were significantly correlated.

Water temperature at the time of autopsy was not correlated with diameters of interrenal cell nuclei, but was correlated positively (but not significantly) with numbers of interrenal nuclei per defined area. Temperatures and small lymphocytes per thousand blood cells were not correlated. However, numbers of small lymphocytes per hundred leucocytes were positively correlated with temperatures. Conversely, percentages of large lymphocytes and neutrophils were negatively correlated with temperatures. Thrombocyte counts were not significantly correlated with water temperatures. A positive correlation existed between water temperatures and hematocrits.

B. CONTINUOUS-LIGHT EXPERIMENTS

Histology of the Head Kidney and Interrenal Gland

Head kidneys of the January 27, 1969 stock tank sample (water temp. 9°C.) were moderately vascularized. Interrenal tissue was moderately to

very hyperplastic. Interrenal cells within many clusters were somewhat atrophic, with unevenly stained, vacuolated cytoplasm. Nucleoli were obvious, but not prominent. No mitotic figures were observed.

Head kidneys of both the March 15, 1969 stock sample (water temp. 4.5°C.) and the April 5, 1969 stock sample (water temp. 5°C.) were extensively vascularized. Interrenal tissue was very hyperplastic. In both samples, a general hypertrophy of interrenal cells compared with the first stock sample was evident. Nucleoli were very prominent. Cytoplasm appeared evenly stained and non-vacuolated. Mitotic figures within the interrenal tissue were observed infrequently.

Head kidneys of the 1, 2 and 4 day control samples were moderately vascularized. Interrenal tissue was only moderately hyperplastic. As in the January 27, 1969 stock sample interrenal cells were frequently atrophic. Mitotic figures were not observed.

Head kidneys and interrenal tissue of fish maintained in continuous light for 1 day and corresponding controls were similar to the 1, 2 or 4 day-control samples. However, mitotic figures within the interrenal tissue of both samples were observed infrequently.

Interrenal tissue of fish maintained in continuous light for 2 days and corresponding controls was moderately to very hyperplastic. Interrenal cells were generally hypertrophied; no signs of atrophy were evident. In both samples, mitotic figures were observed infrequently.

Head kidneys of fish exposed to continuous light for 4 or 8 days and corresponding controls were moderately to well vascularized. Interrenal cells were even more hypertrophied than the previous sample, and without signs of atrophy. Nucleoli were prominent. In all samples, mitotic figures were observed infrequently.

In the 14 day-continuous-light experiment, head kidneys and interrenal tissue of control fish resembled that of the previous samples. However, interrenal tissue of fish exposed to 14 days of continuous light, although hyperplastic, displayed some signs of atrophy. Interrenal cells were less hypertrophied than controls. In several clusters, cytoplasm of cells stained unevenly and was vacuolated.

Mean diameters of the interrenal cell nuclei for all samples of fish involved in the continuous-light experiments are presented in Figure 59 and Table XV. According to Scheffe's test, mean interrenal nuclear diameters (I.N.D.'s) for both the March 15, 1969 and April 5, 1969 stock samples were very significantly (p< 0.01) larger than the mean nuclear diameter for the January stock sample. However, mean diameters for the March 15, 1969 and April 5, 1969 stock samples did not differ significantly.

I.N.D.'s for the 1, 2, 4 and 8 day-control or 1 day-light samples did not differ significantly from the mean nuclear diameter of the January stock sample. The mean diameter for the 2 day control fish was slightly but insignificantly larger than that for the 1 day-control sample, while the mean interrenal nuclear diameter of the 4 day-control sample was significantly larger than that for the 2 day-control sample.

When results for the continuous-light experiments were compared, no consistent differences in mean interrenal nuclear diameters between fish maintained in continuous light and corresponding controls was found. I.N.D.'s for fish maintained in continuous light for 2 or 4 days did not differ significantly from corresponding controls, whereas nuclear diameters of experimental fish in the 1 and 8 day-continuous-light experiments were significantly larger than respective controls, while the mean nuclear diameter of experimental fish in the 14 day-light experiment was significantly

TABLE XV

MEAN INTERRENAL NUCLEAR DIAMETERS (I.N.D.) AND NUMBER OF INTERRENAL NUCLEI
PER DEFINED AREA OF INTERRENAL TISSUE (NO.NU.) OF JUVENILE COHO SALMON
INVOLVED IN THE CONTINUOUS-LIGHT EXPERIMENTS

Number of fish	Sample	Water Temp. (°C.)	I.N.D. (microns) Mean ± S.E.	NO.NU. Mean ± S.E.
6	Jan. 27/69 Stock	9.0	6.40 ± 0.02	27.2 ± 0.7
6	March 15/69 Stock	4.5	6.72 ± 0.02	22.8 ± 1.2
6	April 5/69 Stock	5.0	6.68 ± 0.02	23.9 ± 0.5
4	Jan. 28/69 1 Day Control	9.0	6.24 ± 0.02	25.3 ± 0.8
4	Jan. 29/69 2 Day Control	9.0	6.35 ± 0.02	26.8 ± 0.3
4	Jan. 31/69 4 Day Control	9.5	6.53 ± 0.02	25.3 ± 1.1
4	Feb. 8/69 1 Day Contin. Light	9.5	6.48 ± 0.02	26.6 ± 1.0
4	Feb. 8/69 8 Day Control	9.5	6.24 ± 0.02	25.1 ± 0.5
4	Feb. 17/69 2 Days Contin. Light	4.0	6.59 ± 0.02	23.1 ± 0.8
4	Feb. 17/69 9 Day Control	4.0	6.61 ± 0.02	24.6 ± 0.6
4	Feb. 28/69 4 Days Contin. Light	7.5	6.70 ± 0.02	22.3 ± 0.2
4	Feb. 28/69 11 Day Control	7.5	6.82 ± 0.03	20.5 ± 0.4
4	March 15/69 8 Days Contin. Light	4.5	6.95 ± 0.03	22.1 ± 0.3
4	March 15/69 15 Day Control	4.5	6.74 ± 0.03	23.5 ± 0.7
4	April 5/69 14 Days Contin. Light	5.0	6.56 ± 0.02	24.6 ± 0.6
4	April 5/69 21 Day Control	5.0	6.75 ± 0.03	22.6 ± 1.0

FIGURE 59:   Effect of continuous light on nuclear diameters of
             interrenal cells, and on small-lymphocyte, thrombocyte
             and neutrophil counts in juvenile coho salmon.
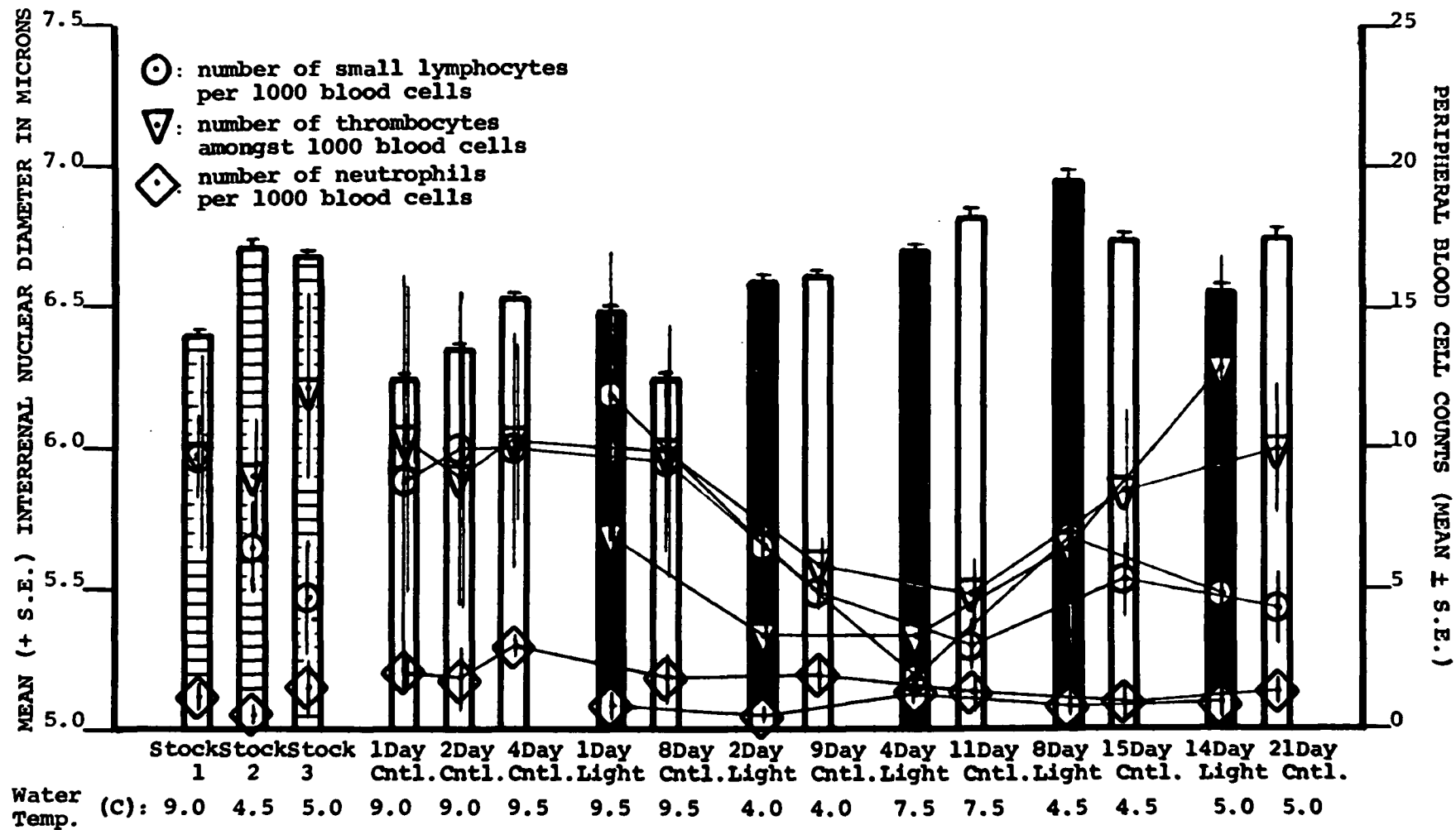
FIGURE 60:   Interrenal cell size* in juvenile coho salmon from the continuous-light experiments.
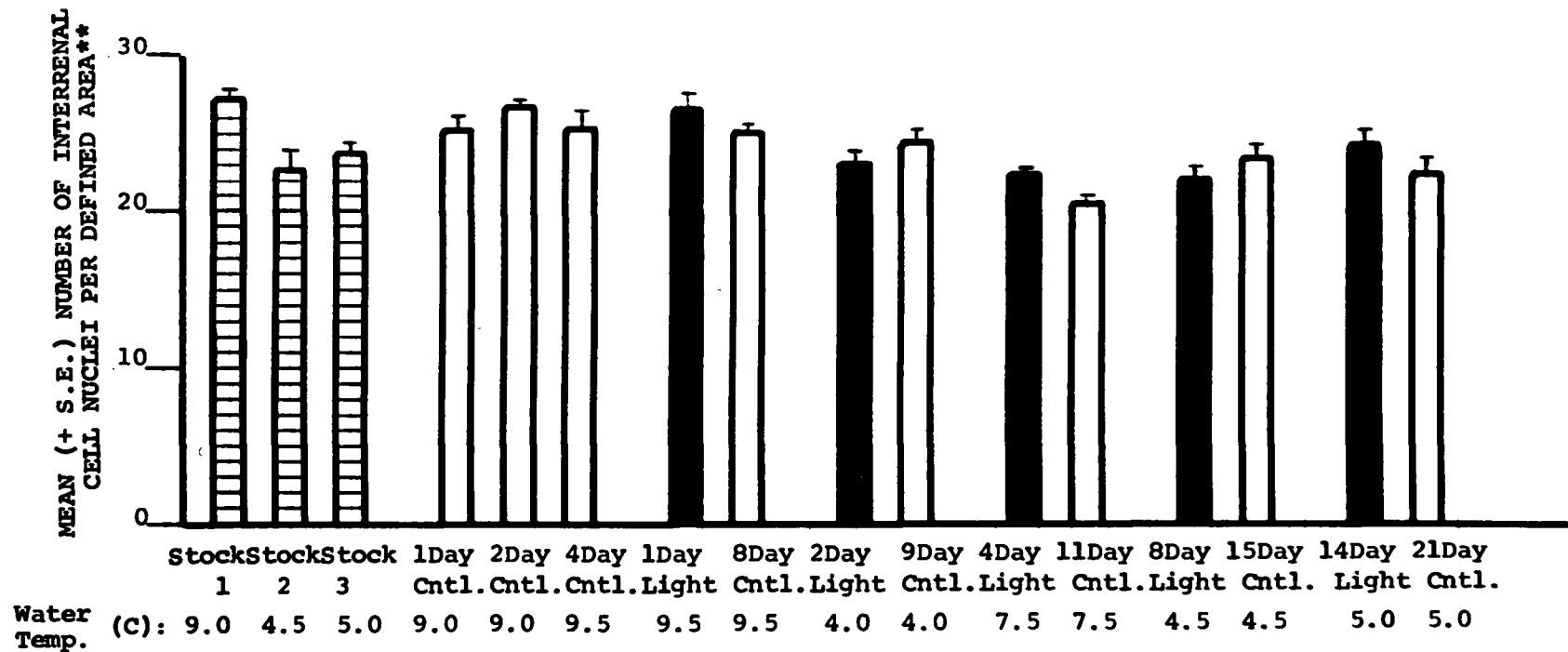
*Interrenal cell size is inversely related to the
 number of interrenal cell nuclei per defined area.

**Defined area is 450 μ^2 of interrenal tissue.

smaller than the control value. Mean diameters of interrenal cell nuclei for fish sampled from warmer waters (9.0-9.5°C.) were consistently smaller than nuclear diameters for fish sampled from colder waters.

The mean interrenal cell size for each sample is represented in Figure 60 and Table XV. Mean numbers of interrenal cell nuclei per defined area for fish exposed to varying periods of continuous light never differed significantly from values for corresponding controls according to Scheffe's test for multiple comparisons. Fish sampled from warmer waters (9.0-9.5°C) consistently exhibited an increase in mean numbers of interrenal nuclei per defined area of interrenal tissue (decrease in cell size) when compared with samples from cooler waters.

Mean nuclear diameters of epsilon cells in the rostral pars distalis of the pituitary gland were determined only for the 4 day-continuous light sample and for the corresponding control sample. Mean values were 5.92 ± 0.94 for the experimental fish, and 5.95 ± 0.04 μ for the control sample. These values are very significantly larger than mean diameters of epsilon cells for all other samples from previous experiments.

Mean Blood Cell Counts

The mean blood cell counts for samples of juvenile coho involved in the continuous-light experiments are presented in Table XVI. Mean numbers of small lymphocytes per thousand blood cells (S.L.'s/1000) were low for all samples (see Fig. 59 and Table XVI) compared with samples from the continuous-darkness experiments and from Bertrand Creek. The mean number of small lymphocytes per thousand blood cells for the first stock-tank sample (water temp. 9.0°C.) was larger than values for the following two stock samples (water temps. 4.5°C. and 5.0°C. respectively). However, these

4	2 Days Continuous Light	4.0	905.0 (10.3)	87.8 (9.4)	6.5 (2.2)	91.5 (3.3)	0.3 (0.3)	1.5 (1.2)	0.5 (0.3)	7.0 (2.2)	**3.3** (1.4)	22.4 (1.6)
4	9 Day Control	4.0	929.8 (21.5)	63.5 (22.4)	4.8 (0.5)	69.3 (7.6)	0.0	3.0 (1.2)	2.0 (0.6)	27.8 (6.6)	5.8 (1.0)	25.1 (1.4)
4	4 Days Continuous Light	7.5	914.5 (4.9)	82.5 (4.3)	1.8 (0.6)	59.0 (3.9)	0.0	5.0 (0.7)	1.3 (0.5)	35.5 (4.0)	3.3 (1.7)	-
4	11 Day Control	7.5	905.8 (11.7)	90.0 (10.8)	3.0 (0.9)	66.3 (4.5)	0.0	2.8 (0.8)	1.3 (0.6)	30.8 (4.4)	4.8 (1.3)	-
4	8 Days Continuous Light	4.5	925.0 (5.9)	70.0 (6.5)	6.8 (2.2)	81.0 (3.1)	0.0	1.0 (0.4)	0.8 (0.3)	18.0 (2.9)	6.5 (3.7)	22.9 (1.0)
4	15 Day Control	4.5	931.3 (16.6)	62.3 (16.3)	5.3 (1.3)	66.0 (9.3)	0.3 (0.3)	4.3 (2.2)	1.0 (0.7)	29.8 (7.4)	8.5 (2.9)	-
4	14 Days Continuous Light	5.0	931.3 (8.1)	63.0 (9.3)	4.8 (1.0)	82.8 (4.2)	0.0	2.3 (0.3)	1.0 (0.6)	14.5 (3.9)	12.8 (4.1)	22.1 (1.0)
4	21 Day Control	5.0	893.3 (32.3)	96.3 (29.5)	4.3 (1.3)	82.5 (5.0)	0.0	0.8 (0.3)	1.3 (0.5)	16.8 (5.0)	10.0 (2.4)	24.5 (0.9)

TABLE XVI

BLOOD CELL COUNTS OF JUVENILE COHO SALMON INVOLVED IN THE
CONTINUOUS-LIGHT EXPERIMENTS

No. of Fish	Treatment/ gm.wt./day for 7 Days	Water Temp. (°C.)	Mat. R.B.C.'s/ 1000	Imm. R.B.C.'s/ 1000	S.L.'s/ 1000	% S.L.'s	L.L.'s/ 1000	% L.L.'s	Nt.'s/ 1000	% Nt.'s	Throm.	Hct.
					MEAN ± S.E.							
6	Stock 1	9.0	952.5 (20.2)	36.5 (19.4)	9.7 (1.5)	77.2 (3.2)	0.2 (0.2)	2.0 (0.5)	1.2 (0.5)	20.8 (3.3)	9.8 (3.5)	21.9 (0.9)
6	Stock 2	4.5	907.3 (9.0)	85.7 (9.6)	6.5 (1.6)	85.7 (3.7)	0.0	0.7 (0.3)	0.5 (0.3)	13.3 (3.4)	9.0 (2.1)	25.2 (0.8)
6	Stock 3	5.0	914.5 (24.2)	81.0 (22.8)	4.7 (2.0)	74.7 (4.2)	0.0	2.2 (0.8)	1.5 (1.0)	22.8 (4.1)	12.2 (3.3)	22.9 (1.4)
4	1 Day Control	9.0	933.8 (11.0)	55.5 (10.6)	8.8 (7.4)	74.5 (7.2)	0.0	3.3 (1.8)	2.0 (0.6)	22.3 (6.2)	10.3 (5.5)	20.6 (2.6)
4	2 Day Control	9.0	915.8 (51.2)	72.5 (52.5)	10.0 (5.5)	73.8 (7.1)	0.0	3.0 (1.5)	1.8 (1.1)	23.3 (6.1)	9.0 (4.7)	20.6 (1.1)
4	4 Day Control	9.5	945.3 (27.1)	41.8 (23.4)	10.0 (4.1)	73.0 (9.9)	0.0	2.5 (1.3)	3.0 (0.4)	24.3 (8.6)	10.3 (3.0)	22.4 (0.4)
4	1 Day Continuous Light	9.5	918.0 (14.8)	69.3 (18.4)	11.8 (5.1)	92.0 (2.4)	0.3 (0.3)	0.8 (0.5)	0.8 (0.5)	7.3 (2.1)	6.8 (2.6)	-
4	8 Day Control	9.5	908.5 (9.3)	79.8 (9.0)	9.5 (3.2)	76.3 (8.7)	0.5 (0.3)	2.8 (1.5)	1.8 (0.9)	20.8 (7.4)	9.8 (4.5)	-

differences were not significant ($p > 0.05$) according to Student's t test. S.L.'s/1000 for the 1, 2 and 4 day-control samples (water temp. 9.0-9.5°C.) and for fish receiving 1 day of continuous light and corresponding controls (water temp. 9.5°C.) were similar to the value for the first stock sample. Within these samples, counts between individual fish were extremely variable (high S.E.'s). Mean numbers of small lymphocytes per thousand blood cells were very low for fish maintained in continuous light for 2, 4, 8 and 14 days, and for corresponding control samples (water temperatures 4.0-7.5°C. at time of sacrifice). No differences between samples were significant according to Student's t test. However, S.L.'s/1000 were slightly larger for fish maintained in continuous light for 1, 2, 8 or 14 days compared with corresponding control values.

Thrombocyte counts for samples involved in the continuous-light experiments did not differ significantly. Mean numbers of thrombocytes amongst one thousand blood cells were similar for 1, 2, 4 and 8 day-controls, and for the first stock sample (water temps. 9.0-9.5°C.). However, thrombocyte counts between individual fish within these samples were extremely variable (high S.E.'s). Mean thrombocyte counts for both controls and experimental fish from the 2, 4 and 8 day-light experiments (water temps. 4.0-7.5°C.) were low, although mean counts for the 14 day-light experiment increased.

Mean numbers of large lymphocytes per thousand blood cells were negligible for all samples. Mean numbers of neutrophils per thousand blood cells were small for all samples (Table XVI and Fig. 59), and did not differ significantly for any comparisons. Mean hematocrits for each sample also did not differ significantly.

Correlation Coefficients of Variables Measured in the Continuous-Light
Experiments

Correlation coefficients for all variables measured from juvenile
coho involved in the continuous-light experiments are presented in Table
XVII. Summary statistics for each variable are presented in Table VI of
Appendix II.

As in previous experiments, interrenal nuclear diameters (I.N.D.'s)
and numbers of interrenal nuclei per defined area of interrenal tissue
(No. Nu.'s) were negatively correlated. I.N.D.'s were negatively correlated
with numbers of small lymphocytes per thousand blood cells (S.L.'s/1000),
while No. Nu.'s were positively correlated with S.L.'s/1000. Thrombocyte
counts were not correlated with either interrenal nuclear diameters or
numbers of interrenal nuclei per defined area of interrenal tissue.

Numbers of small lymphocytes per thousand blood cells were
positively correlated with both numbers of small lymphocytes per hundred
leucocytes and thrombocyte counts. Thrombocyte counts were also positively
correlated with numbers of neutrophils per thousand blood cells and numbers
of small lymphocytes per hundred leucocytes. S.L.'s/1000 were not correlated
significantly with either numbers of large lymphocytes per thousand blood
cells or numbers of neutrophils per thousand blood cells. Large lymphocytes
per thousand cells and large lymphocytes per hundred leucocytes were not
correlated. However, numbers of neutrophils per thousand blood cells and
numbers of neutrophils per hundred leucocytes were positively correlated.

Water temperature at the time of autopsy was negatively correlated
with nuclear diameters of interrenal cells, and positively correlated with
numbers of interrenal nuclei per defined area of interrenal tissue.
Temperatures and numbers of small lymphocytes per thousand blood cells were

TABLE XVII

CORRELATION MATRIX OF VARIABLES MEASURED FROM JUVENILE COHO SALMON INVOLVED IN THE CONTINUOUS-LIGHT EXPERIMENTS

	Nt.'s/1000	% Nt.'s	Imm. R.B.C.'s/ 1000	THROM.	I.N.D.	No.Nu.
Length (cm.)	-0.089	-0.293*	-0.018	0.036	0.158	-0.181
Weight (gm.)	-0.032	-0.261*	0.064	-0.007	0.155	-0.162
Hematocrit	-0.071	-0.224	-0.034	0.019	0.512**	-0.320*
Temperature	0.239	0.087	-0.096	0.021	-0.607**	0.326**
S.L.'s/1000	0.135	-0.459**	-0.078	0.505**	-0.275*	0.272*
% S.L.'s	-0.427**	-0.992**	0.009	0.284*	-0.025	0.002
L.L.'s/1000	-0.038	-0.101	0.134	-0.044	-0.198	0.163
% L.L.'s	0.201	0.615**	0.017	-0.319*	-0.094	0.056
Nt.'s/1000	1.000	0.448**	-0.090	0.287*	-0.149	0.186
% Nt.'s		1.000	-0.016	-0.260*	0.044	-0.009
Imm. R.B.C.'s/1000			1.000	-0.045	0.018	-0.008
Thrombocytes				1.000	0.022	0.041
I.N.D.					1.000	-0.691**
No.Nu.						1.000

*Significant at the 95 per cent level
**Significant at the 99 per cent level

Critical Absolute Values: 5%: 0.250
1%: 0.325

Nt.'s/1000: Neutrophils per 1000 blood cells
% Nt.'s: Neutrophils per 100 Leucocytes
Imm. R.B.C.'s/1000: Immature Erythrocytes per 1000 Blood Cells
THROM.: Thrombocytes amongst 1000 blood cells

I.N.D.: Interrenal Nuclear Diameter
No.Nu.: Number of Interrenal Nuclei per Defined Area of Interrenal Tissue

TABLE XVII

CORRELATION MATRIX OF VARIABLES MEASURED FROM JUVENILE COHO SALMON
INVOLVED IN THE CONTINUOUS-LIGHT EXPERIMENTS

	LENGTH	WEIGHT	HEMATOCRIT	TEMPERATURE	S.L.'s/1000	% S.L.'s	L.L.'s/1000	% L.L.'s
Length (cm.)	1.000	0.918**	0.345**	-0.138	0.089	0.301*	-0.139	-0.252*
Weight (gm.)		1.000	0.364**	-0.185	0.037	0.273*	-0.057	-0.253*
Hematocrit			1.000	-0.429**	0.030	0.268	0.002	-0.423**
Temperature				1.000	0.299*	-0.100	0.185	0.127
S.L.'s/1000					1.000	0.464**	0.024	-0.337**
% S.L.'s						1.000	0.107	-0.710**
L.L.'s/1000							1.000	-0.090
% L.L.'s								1.000
Nt.'s/1000								
% Nt.'s								
Imm. R.B.C.'s/1000								
Thrombocytes								
I.N.D.								
No.Nu.								

*Significant at the 95 per cent level   Critical Absolute Values: 5%: 0.250

**Significant at the 99 per cent level   1%: 0.325

S.L.'s/1000: Small Lymphocytes per 1000 blood cells   L.L.'s/1000: Large Lymphocytes per 1000 blood cells

% S.L.'s: Small Lymphocytes per 100 Leucocytes   % L.L.'s: Large Lymphocytes per 100 Leucocytes

Temperature = Water Temperature at time of Autopsy

positively correlated. However, temperatures were not correlated significantly with either the percentages of small lymphocytes, large lymphocytes or neutrophils per hundred leucocytes. Thrombocyte counts were not correlated with water temperatures. A negative correlation existed between water temperatures and hematocrits.

C. FLASHING-LIGHT EXPERIMENTS
Histology of the Head Kidney and Interrenal Gland

Head kidneys of both the April 7, 1969 stock-tank sample (water temp. 5°C.) and May 31, 1969 stock sample (water temp. 9°C.) were moderately to well vascularized. In both samples, interrenal tissue was moderately to very hyperplastic. Interrenal cells of fish from the first stock sample were hypertrophied, with enlarged nuclei and prominent nucleoli. Cytoplasm was evenly stained and non-vacuolated. However, in the second stock sample, considerable atrophy of the interrenal tissue was apparent. Nuclei were irregularly shaped, and closely apposed to each other. Nucleoli were not as prominent as in the preceding stock sample. Cytoplasm of interrenal cells often stained unevenly. Intercellular spaces within clusters of interrenal cells were frequently observed. Mitotic figures were infrequently seen within the interrenal tissue of the April 7, 1969 stock sample, but were never observed in interrenal tissue from the May 31, 1969 stock sample.

Interrenal tissues of the 1, 2, 4, 7 and 8 day control samples and the samples of fish exposed to continuously flashing light for twelve hours or 1 day all appeared similar to the interrenal tissues of fish from the first stock tank sample. Interrenal cells were hypertrophied, with no signs of atrophy evident. Interrenal tissue from fish exposed to flashing lights for 2 days and from corresponding controls appeared to be somewhat atrophic.

Within each head kidney, clusters of interrenal cells with irregularly shaped, closely apposed nuclei and unevenly stained cytoplasm were observed. Interrenal cells within other clusters were hypertrophied and healthy in appearance.

Interrenal tissue from fish exposed to continuously flashing lights for 4 days appeared to be generally stimulated, with very few clusters of somewhat atrophic interrenal cells. On the other hand, interrenal cells from corresponding control fish were generally atrophic. Mitotic figures were quite frequently observed within the interrenal tissue of experimental fish, whereas mitotic figures were rarely observed within interrenal tissue of controls.

Interrenal cells within head kidneys from fish exposed to 8 days of flashing lights appeared to be less hypertrophied than previous samples, although interrenal tissue was still hyperplastic. Many clusters of cells appeared to be somewhat atrophic, although nucleoli were still prominent. Mitotic figures were infrequently observed. Interrenal tissue from the corresponding control sample was more atrophic, with considerable shrinkage of cells, displaying inconspicuous nucleoli and unevenly stained cytoplasm. No mitotic figures were observed.

Head kidneys from fish exposed to a continuously flashing light for 16 days and from corresponding controls were not as extensively vascularized as head kidneys from previous samples. Interrenal tissue of both experimental and control fish was extremely atrophic. All interrenal cells were shrunken. Within a cluster of cells, the very closely apposed nuclei were irregularly shaped and densely stained, with very inconspicuous nucleoli. Unlike previous samples, the interrenal tissue was not hyperplastic. No mitotic figures were found within the interrenal tissue of either experimental or

TABLE XVIII

MEAN INTERRENAL NUCLEAR DIAMETERS (I.N.D.) AND NUMBER OF INTERRENAL NUCLEI
PER DEFINED AREA OF INTERRENAL TISSUE (NO.NU.) OF JUVENILE COHO SALMON
INVOLVED IN THE FLASHING LIGHT-EXPERIMENTS

Number of Fish	Sample	Water Temp. (°C.)	I.N.D. (microns) Mean ± S.E.	NO.NU. Mean ± S.E.
4	April 7/69 Stock	5.0	6.61 ± 0.02	22.3 ± 0.3
4	May 31/69 Stock	9.0	6.33 ± 0.03	25.3 ± 0.5
4	April 8/69 1 Day Control	5.5	6.62 ± 0.02	21.7 ± 0.6
4	April 9/69 2 Day Control	5.5	6.54 ± 0.02	23.9 ± 0.5
4	April 11/69 4 Day Control	5.5	6.60 ± 0.02	22.5 ± 1.0
4	April 16/69 12 Hour Flash	6.5	6.58 ± 0.02	21.8 ± 0.7
4	April 16/69 7½ Day Control	6.5	6.82 ± 0.03	18.8 ± 0.3
4	April 25/69 1 Day Flash	6.5	6.75 ± 0.03	20.2 ± 0.8
4	April 25/69 8 Day Control	6.5	6.56 ± 0.03	22.8 ± 0.5
4	May 5/69 2 Day Flash	7.5	6.49 ± 0.02	21.6 ± 0.8
4	May 5/69 9 Day Control	7.5	6.54 ± 0.02	20.1 ± 1.2
4	May 16/69 4 Day Flash	8.0	6.53 ± 0.02	22.6 ± 0.2
4	May 16/69 11 Day Control	8.0	6.43 ± 0.02	24.5 ± 0.5
4	May 31/69 8 Day Flash	9.0	6.33 ± 0.03	23.0 ± 1.1
4	May 31/69 15 Day Control	9.0	6.19 ± 0.02	24.6 ± 0.9
4	June 23/69 16 Day Flash	9.5	6.01 ± 0.02	28.9 ± 1.0
4	June 23/69 23 Day Control	9.5	5.95 ± 0.02	30.5 ± 0.6

FIGURE 61:   Effect of continuously flashing lights on nuclear

diameters of interrenal cells, and on hematocrits

and small-lymphocyte counts in juvenile coho salmon.
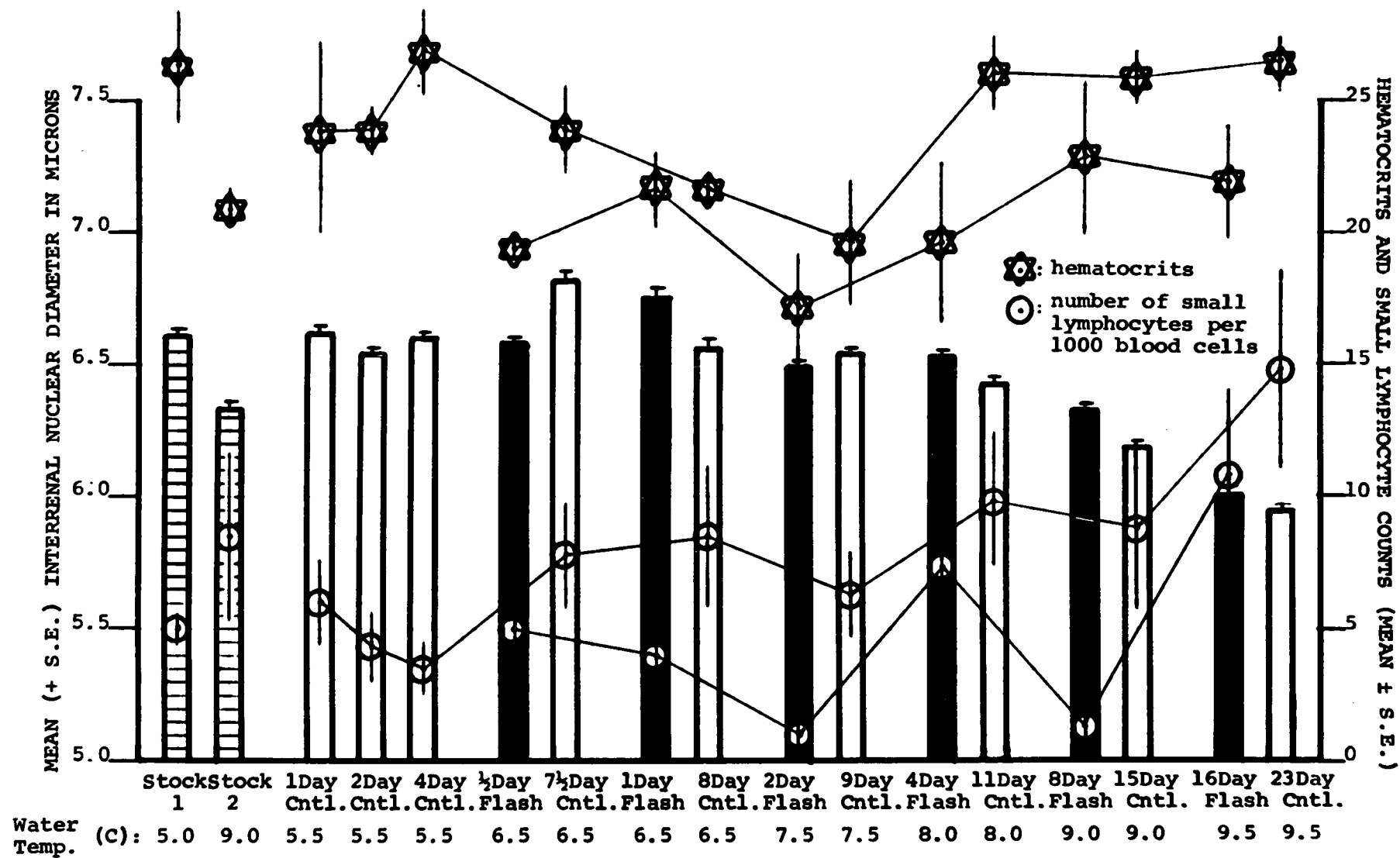
FIGURE 62:   Effect of continuously flashing lights on nuclear

diameters of interrenal cells, and on thrombocyte and

neutrophil counts in juvenile coho salmon.
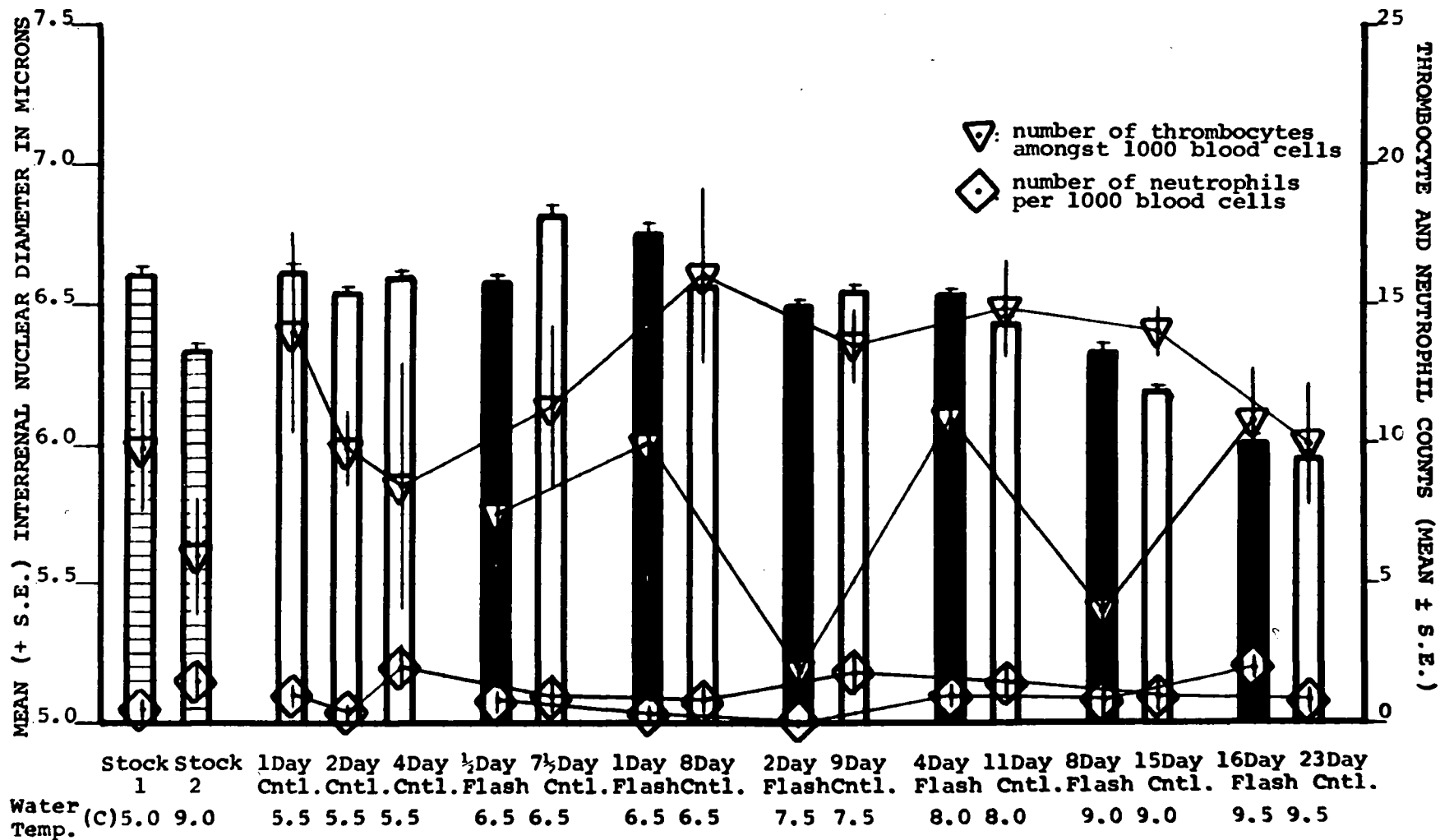
FIGURE 63:   Interrenal cell size* in juvenile coho salmon from the
              continuously flashing-light experiments.
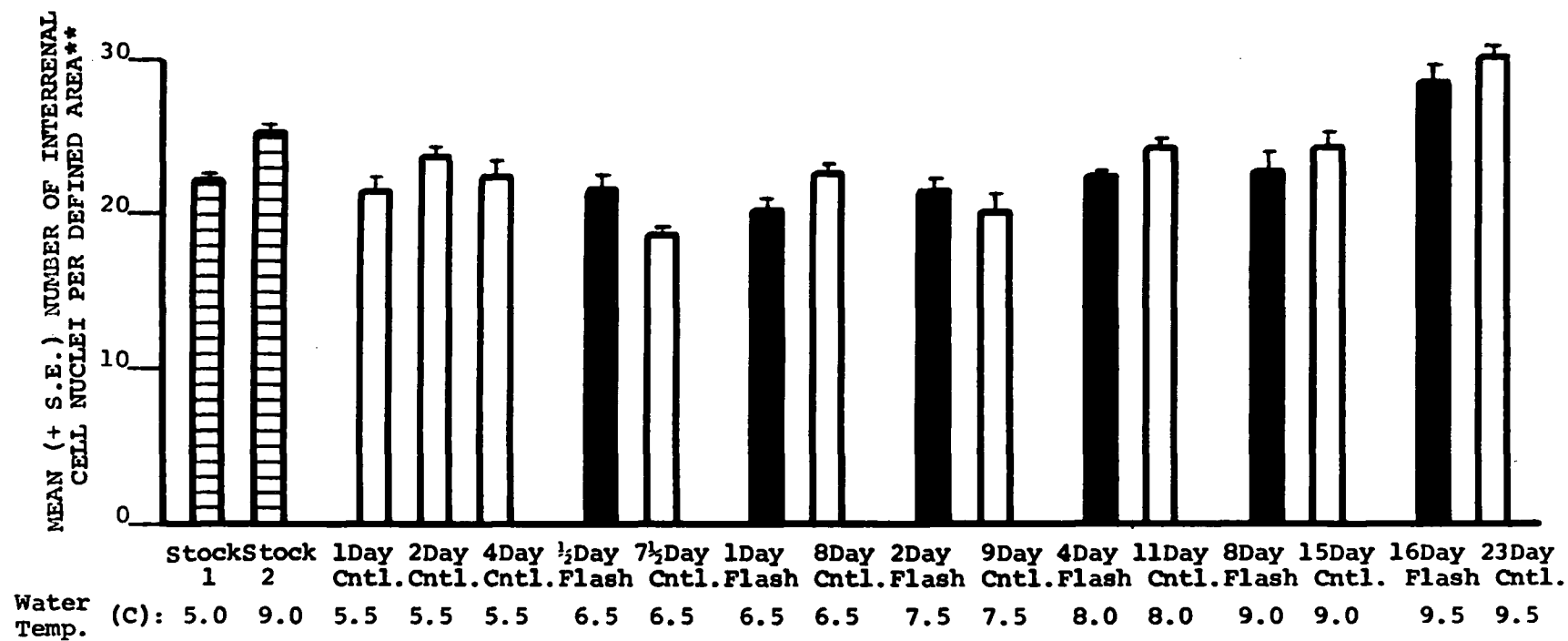
*Interrenal cell size is inversely related to the number of interrenal cell nuclei per defined area.

**Defined area is 450 μ^2 of interrenal tissue.

control head kidneys.

The mean diameters of interrenal cell nuclei for all samples of fish involved in the flashing-light experiments are presented in Figures 61 and 62 and Table XVIII. The mean interrenal nuclear diameter for the April 7, 1969 stock sample was very significantly ($p < 0.01$) larger than the mean value for the May 31, 1969 stock sample according to Scheffe's test. Mean interrenal nuclear diameters (I.N.D.'s) for the 1, 2, 4, 8, 9 or 11 day controls did not differ significantly from the mean diameter of interrenal nuclei for the April 7, 1969 stock sample, although the mean diameter for the 7 day control sample was very significantly larger. I.N.D.'s for samples of fish exposed to flashing lights for twelve hours, 1, 2 or 4 days did not differ significantly from the mean nuclear diameter for the April 7, 1969 stock sample. However, mean diameters of interrenal cell nuclei for fish exposed to continuously flashing lights for 8 and 16 days, and for corresponding controls were very significantly smaller than the I.N.D. for the first stock sample.

Mean diameters of interrenal cell nuclei for the 1, 2, 4, 7, 8 and 9 day-control samples and for the twelve hour, 1, 2 and 4 day-flash samples all were very significantly larger than the mean diameter for the May 31, 1969 stock sample. However, mean I.N.D.'s for the 11 day control sample and for both the 8 day-flash and control fish sampled on May 31, 1969 did not differ significantly from the value for this stock sample. Nuclear diameters for fish exposed to continuously flashing lights for 16 days and for corresponding controls were very significantly smaller than the mean interrenal nuclear diameter for the second stock sample.

The mean interrenal nuclear diameter for the fish exposed to flashing lights for twelve hours was significantly smaller than the

corresponding control value, whereas the mean nuclear diameter for fish from the 1 day-flash experiment was significantly larger than the I.N.D. for controls. In the other experiments, mean diameters of interrenal cell nuclei for fish exposed to varying periods of flashing lights did not differ significantly from values for corresponding controls. However, mean nuclear diameters for fish exposed to 4, 8 or 16 days of continuously flashing lights were larger than nuclear diameters for corresponding control samples.

The mean interrenal cell size for each sample is represented in Figure 63 and Table XVIII. The mean number of interrenal cell nuclei per defined area of interrenal tissue (No. Nu.) for the first stock sample did not differ significantly from the mean value for the second stock sample according to Scheffe's test. However, interrenal cells for the first stock sample were larger (smaller No. Nu.). The mean number of interrenal nuclei per defined area for the first stock sample was very significantly smaller than mean values for fish exposed to flashing lights for 16 days and for corresponding controls, but did not differ significantly from other samples. No. Nu's for the 16 day-flash and corresponding control samples were very significantly larger than mean values for all previous samples excluding the second stock sample. In each experiment, mean numbers of interrenal nuclei per defined area for fish exposed to flashing lights never differed significantly from corresponding control values. However, interrenal cells of fish exposed to 4, 8 or 16 days of continuously flashing lights were larger (smaller No. Nu.'s) than values for corresponding controls.

Mean Blood Cell Counts

Mean blood cell counts for samples of juvenile coho involved in the flashing-light experiments are presented in Table XIX. The mean number of

4	2 Day Flash	7.5	946.0 (12.2)	52.5 (12.1)	1.0 (0.4)	83.5 (3.7)	0.0	1.8 (0.5)	0.0	14.8 (3.2)	1.8 (0.8)	17.1 (2.1)
4	9 Day Control	7.5	943.0 (5.1)	48.8 (6.9)	6.3 (1.6)	68.0 (17.0)	0.3 (0.3)	5.8 (4.8)	1.8 (0.8)	26.3 (3.9)	13.5 (1.3)	19.6 (2.3)
4	4 Day Flash	8.0	946.5 (14.5)	45.3 (13.7)	7.3 (1.5)	83.0 (1.9)	0.0	1.0 (0.6)	1.0 (0.4)	14.8 (1.3)	10.8 (1.8)	19.6 (3.0)
4	11 Day Control	8.0	917.5 (8.8)	71.3 (9.9)	9.8 (2.4)	92.3 (1.9)	0.0	0.5 (0.5)	1.5 (0.6)	7.3 (1.8)	14.8 (1.7)	26.0 (1.4)
4	8 Day Flash	9.0	972.0 (8.7)	26.0 (8.2)	1.3 (0.8)	68.0 (7.9)	0.0	4.0 (1.8)	0.8 (0.8)	28.0 (6.5)	4.0 (1.9)	22.8 (2.9)
4	15 Day Control	9.0	966.8 (6.3)	21.5 (6.0)	8.8 (2.0)	83.0 (7.9)	0.5 (0.3)	1.3 (0.9)	1.0 (0.7)	18.3 (8.0)	14.0 (0.9)	25.8 (1.0)
4	16 Day Flash	9.5	939.5 (16.2)	47.5 (19.5)	10.8 (3.3)	88.5 (3.8)	0.3 (0.3)	0.5 (0.3)	2.0 (0.4)	11.0 (3.6)	10.8 (1.9)	21.9 (2.1)
4	23 Day Control	9.5	968.8 (5.1)	15.8 (6.5)	14.8 (3.8)	80.8 (5.5)	0.0	2.0 (1.4)	0.8 (0.5)	17.3 (5.7)	10.0 (2.2)	26.5 (0.8)

TABLE XIX

BLOOD CELL COUNTS OF JUVENILE COHO SALMON INVOLVED IN THE
FLASHING-LIGHT EXPERIMENTS

— MEAN ± S.E. —

No. of Fish	Treatment/ gm.wt./day for 7 Days	Water Temp. (°C.)	Mat. R.B.C.'s/ 1000	Imm. R.B.C.'s/ 1000	S.L.'s/ 1000	% S.L.'s	L.L.'s/ 1000	% L.L.'s	Nt.'s/ 1000	% Nt.'s	Throm.	Hct.
4	Stock 1	5.0	973.5 (3.5)	21.0 (3.0)	5.0 (0.6)	89.8 (2.4)	0.0	1.5 (0.5)	0.5 (0.3)	8.8 (2.3)	9.8 (2.1)	26.3 (2.1)
4	Stock 2	9.0	969.0 (7.7)	18.5 (9.0)	8.5 (3.2)	79.8 (6.7)	0.0	0.8 (0.5)	1.5 (0.7)	19.5 (6.8)	6.0 (2.1)	20.8 (0.4)
4	1 Day Control	5.5	969.8 (9.7)	23.3 (10.3)	6.0 (1.6)	83.8 (7.0)	0.0	1.3 (0.6)	1.0 (0.4)	15.0 (6.4)	14.0 (3.6)	23.6 (3.6)
4	2 Day Control	5.5	959.5 (3.4)	35.8 (2.6)	4.3 (1.3)	77.5 (8.7)	0.3 (0.3)	0.8 (0.3)	0.3 (0.3)	21.8 (8.7)	9.8 (1.3)	23.8 (0.9)
4	4 Day Control	5.5	977.5 (3.1)	17.0 (2.7)	3.5 (1.0)	70.0 (3.3)	0.0	1.0 (0.6)	2.0 (0.7)	29.0 (3.0)	8.5 (4.4)	26.8 (1.6)
4	12 Hour Flash	6.5	932.3 (7.8)	62.5 (6.2)	5.0 (1.7)	87.5 (1.9)	0.0	0.5 (0.3)	0.3 (0.3)	11.5 (2.0)	7.5 (1.9)	19.3 (0.3)
4	7.1/2 Day Control	6.5	972.8 (6.1)	18.5 (7.6)	7.8 (2.0)	85.0 (1.9)	0.0	0.3 (0.3)	1.0 (0.7)	14.8 (1.9)	11.3 (2.9)	23.9 (1.6)
4	1 Day Flash	6.5	944.3 (5.1)	51.3 (5.6)	4.0 (1.3)	84.0 (2.4)	0.0	1.5 (0.7)	0.3 (0.3)	14.0 (2.5)	10.0 (4.7)	21.6 (1.4)
4	8 Day Control	6.5	933.5 (9.6)	57.0 (7.2)	8.5 (2.7)	82.8 (1.7)	0.3 (0.3)	0.5 (0.3)	0.8 (0.5)	16.5 (1.7)	16.0 (3.1)	21.6 (0.6)

small lymphocytes per thousand blood cells for the first stock-tank sample (water temp. 5.0°C.) was smaller than the mean value for the second stock sample (water temp. 9.0°C.). However, this difference was not significant according to Student's t test. Values for 1, 2 and 4 day-control samples were similar to the first stock sample (see Fig. 61 and Table XIX). S.L.'s/ 1000 for succeeding control samples generally increased with warmer water temperatures. S.L.'s/1000 for the 1, 2 and 4 day-control samples and for the first stock sample were very significantly smaller than the mean value for the 23 day control sample. Mean small-lymphocyte counts for samples of fish exposed to varying periods of flashing lights were smaller than corresponding control values for all experiments. However, these differences were only significant for the 2 and 8 day-flash experiments.

Mean thrombocyte counts for control samples did not differ significantly from one another. However, mean values for samples of fish exposed to varying periods of flashing lights were extremely variable (Fig. 62 and Table XIX). Furthermore, mean thrombocyte counts for samples of fish exposed to continuously flashing lights for twelve hours, 1, 2, 4 and 8 days were smaller than values for corresponding controls. However, differences were only significant for the 2 and 8 day-flashing light experiments.

Mean numbers of large lymphocytes per thousand blood cells were negligible for all samples. Mean numbers of neutrophils per thousand blood cells were low for all samples (Fig. 62 and Table XIX).

Mean hematocrit values for each sample are presented in Figure 61 and Table XIX. Hematocrits for samples of fish exposed to twelve hours, 2, 4, 8 and 16 days of continuously-flashing lights were lower than values for corresponding controls. However, these differences were not significant according to Student's t test.

Correlation Coefficients of Variables Measured in the Flashing-Light
Experiments

A correlation matrix for all variables measured from juvenile coho
salmon involved in the continuously flashing-light experiments is presented
in Table XX. Summary statistics for each variable are presented in Table
VII of Appendix II.

As in all previous experiments, interrenal nuclear diameters (I.N.D.'s)
and numbers of interrenal nuclei per defined area of interrenal tissue
(No. Nu.'s) were negatively correlated. I.N.D.'s were negatively correlated
with numbers of small lymphocytes per thousand blood cells, whereas No. Nu.'s
were very positively correlated with this variable. Neither I.N.D.'s nor
No. Nu.'s were significantly correlated with either numbers of large
lymphocytes or numbers of neutrophils per thousand blood cells. Furthermore,
diameters of interrenal cell nuclei or numbers of interrenal nuclei per
defined area were not significantly correlated with numbers of small
lymphocytes, large lymphocytes or neutrophils per hundred leucocytes, or with
thrombocyte counts.

As in all previous experiments, numbers of small lymphocytes per
thousand blood cells (S.L.'s/1000) were positively correlated with numbers
of small lymphocytes per hundred leucocytes, and with numbers of thrombocytes
amongst one thousand blood cells. Thrombocyte counts were also positively
correlated with numbers of large lymphocytes and neutrophils per thousand
blood cells. S.L.'s/1000 were not correlated with numbers of large
lymphocytes per thousand blood cells, but were positively correlated with
numbers of neutrophils per thousand blood cells. Numbers of large lymphocytes
per thousand blood cells and numbers of large lymphocytes per hundred
leucocytes were positively correlated. Neutrophils per thousand blood cells

TABLE XX

CORRELATION MATRIX OF VARIABLES MEASURED FROM JUVENILE COHO SALMON
INVOLVED IN THE FLASHING-LIGHT EXPERIMENTS

	Nt.'s/1000	% Nt.'s	Imm. R.B.C.'s/1000	THROM	I.N.D.	No.Nu.
Length (cm.)	-0.194	-0.198	-0.104	-0.048	-0.302*	0.285*
Weight (gm.)	-0.189	-0.205	-0.104	-0.039	-0.323*	0.366**
Hematocrit	0.028	-0.047	-0.237	0.056	-0.027	0.174
Temperature	0.164	0.027	-0.069	-0.111	-0.707**	0.581**
S.L.'s/1000	0.346**	-0.353**	-0.173	0.503**	-0.350**	0.405**
% S.L.'s	-0.234**	-0.971**	0.230	0.104	-0.098	0.233
L.L.'s/1000	0.147	0.211	0.076	0.272*	-0.058	0.014
% L.L.'s	0.071	0.391**	-0.031	-0.114	-0.025	-0.183
Nt.'s/1000	1.000	0.237	-0.131	0.302*	-0.050	0.039
% Nt.'s		1.000	-0.267*	-0.075	0.088	-0.191
Imm. R.B.C.'s/1000			1.000	0.016	0.139	-0.151
Thrombocytes				1.000	0.044	-0.010
I.N.D.					1.000	-0.775**
No.Nu.						1.000

*Significant at the 95 per cent level
**Significant at the 99 per cent level

Critical Absolute Values:　5%:　0.250
　　　　　　　　　　　　　　1%:　0.325

Nt.'s/1000:　　Neutrophils per 1000 blood cells
% Nt.'s:　　　Neutrophils per 100 Leucocytes
Imm. R.B.C.'s/
　　1000:　　Immature Erythrocytes per 1000 blood cells
THROM.:　　　Thrombocytes amongst 1000 blood cells

I.N.D.:　Interrenal Nuclear Diameter
No.Nu.:　Number of Interrenal Nuclei per Defined Area of Interrenal Tissue

TABLE XX

CORRELATION MATRIX OF VARIABLES MEASURED FROM JUVENILE COHO SALMON INVOLVED IN THE FLASHING-LIGHT EXPERIMENTS

	LENGTH	WEIGHT	HEMATOCRIT	TEMPERATURE	S.L.'s/1000	% S.L.'s	L.L.'s/1000	% L.L.'s
Length (cm.)	1.000	0.947**	0.341**	0.258*	0.191	0.179	-0.103	0.013
Weight (gm.)		1.000	0.441**	0.331**	0.218	0.175	-0.067	0.065
Hematocrit			1.000	-0.053	0.123	0.078	0.010	-0.093
Temperature				1.000	0.267*	-0.028	0.024	0.097
S.L.'s/1000					1.000	0.346**	0.082	-0.181
% S.L.'s						1.000	-0.257*	-0.575**
L.L.'s/1000							1.000	0.281*
% L.L.'s								1.000
Nt.'s/1000								
% Nt.'s								
Imm. R.B.C.'s/1000								
Thrombocytes								
I.N.D.								
No.Nu.								

*Significant at the 95 per cent level   Critical Absolute Values: 5%: 0.250

**Significant at the 99 per cent level   1%: 0.325

S.L.'s/1000: Small Lymphocytes per 1000 blood cells   L.L.'s/1000: Large Lymphocytes per 1000 blood cells

% S.L.'s: Small Lymphocytes per 100 Leucocytes   % L.L.'s: Large Lymphocytes per 100 Leucocytes

Temperature = Water Temperature at time of Autopsy

and neutrophils per hundred leucocytes were also positively (although not significantly) correlated.

As in the continuous-light experiments and in experiments where fish were autopsied seasonally at Bertrand Creek, water temperatures at the time of autopsy were negatively correlated with diameters of interrenal cell nuclei, and positively correlated with the numbers of interrenal nuclei per defined area. Temperatures were also positively correlated with numbers of small lymphocytes per thousand blood cells. However, water temperatures were not significantly correlated with numbers of small lymphocytes, large lymphocytes or neutrophils per hundred leucocytes, or with thrombocyte counts or hematocrits.

Correlation Coefficients of Variables Measured from all Experiments

A correlation matrix for variables measured in juvenile coho salmon involved in all experiments (excluding injection experiments) is presented in Table XXI. Summary statistics for each variable are found in Table VIII of Appendix II.

Interrenal nuclear diameters (I.N.D.'s) were positively correlated with lengths and weights of fish. Numbers of interrenal nuclei per defined area of interrenal tissue (No. Nu.'s) were negatively correlated with lengths, but were not significantly related to weights. I.N.D.'s and No. Nu.'s were negatively correlated. Both numbers of small lymphocytes per thousand blood cells and numbers of small lymphocytes per hundred leucocytes were negatively correlated with diameters of interrenal nuclei, and positively correlated with numbers of interrenal nuclei per defined area. Neither large lymphocytes per thousand blood cells, neutrophils per thousand blood cells nor thrombocytes amongst one thousand blood cells were significantly

TABLE XXI

CORRELATION MATRIX OF VARIABLES MEASURED FROM JUVENILE COHO SALMON

FROM ALL EXPERIMENTS

	Nt.'s/1000	% Nt.'s	Imm. R.B.C.'s/1000	THROM.	I.N.D.	No.Nu.	**E.N.D.
Length (cm.)	0.160*	0.107	0.173*	0.077	0.422**	-0.274**	0.365**
Weight (gm.)	0.146	0.054	0.165*	0.055	0.277**	-0.108	0.308*
Hematocrit	-0.097	-0.291**	-0.162*	-0.029	-0.187**	0.099	-0.088
Temperature	-0.139*	-0.374**	-0.185**	0.200**	-0.655**	0.416**	-0.524**
S.L.'s/1000	-0.035	-0.505**	-0.007	0.579**	-0.265**	0.198**	-0.357**
% S.L.'s	-0.249**	-0.980**	-0.013	0.323**	-0.273**	0.194**	-0.527**
L.L.'s/1000	-0.006	-0.082	-0.058	0.043	-0.051	-0.020	0.011
% L.L.'s	0.047	0.456**	0.005	-0.289**	0.133	-0.048	0.176
Nt.'s/1000	1.000	0.277**	-0.023	0.136	0.101	0.002	0.275*
% Nt.'s		1.000	0.013	-0.297**	0.271**	-0.195**	0.551**
Imm. R.B.C.'s/1000			1.000	0.045	0.182**	-0.092	0.253
Thrombocytes				1.000	-0.024	-0.068	-0.245
I.N.D.					1.000	-0.640**	0.556**
No.Nu.						1.000	-0.624**
E.N.D.							1.000

*Significant at the 95 per cent level   Critical Absolute Values: 5%: 0.138
**Significant at the 99 per cent level   1%: 0.181

Nt.'s/1000:   Neutrophils per 1000 blood cells   I.N.D.:   Interrenal Nuclear Diameter
% Nt.'s: Neutrophils per 100 Leucocytes   No.Nu.:   Number of Interrenal Nuclei per Defined
Imm. R.B.C.'s/   Area of Interrenal Tissue
    1000:   Immature Erythrocytes per 1000 blood cells   E.N.D.:   Epsilon Cell Nuclear Diameter
THROM.:   Thrombocytes amongst 1000 blood cells   ** Critical Absolute Values for E.N.D.'s: 5%: 0.273
                                                                                1%: 0.354

TABLE XXI

CORRELATION MATRIX OF VARIABLES MEASURED FROM JUVENILE COHO SALMON
FROM ALL EXPERIMENTS

	LENGTH	WEIGHT	HEMATOCRIT	TEMPERATURE	S.L.'s/1000	% S.L.'s	L.L.'s/1000	% L.L.'s
Length (cm.)	1.000	0.941**	-0.188**	-0.374**	-0.064	-0.067	-0.013	-0.068
Weight (gm.)		1.000	-0.176*	-0.257**	-0.043	-0.020	-0.004	-0.071
Hematocrit			1.000	0.455**	0.182**	0.252**	0.077	-0.006
Temperature				1.000	0.460**	0.381**	0.107	-0.238**
S.L.'s/1000					1.000	0.513**	0.123	-0.311**
% S.L.'s						1.000	0.064	-0.612**
L.L.'s/1000							1.000	0.049
% L.L.'s								1.000
Nt.'s/1000								
% Nt.'s								
Imm. R.B.C.'s/1000								
Thrombocytes								
I.N.D.								
No.Nu.								
E.N.D.								

*Significant at the 95 per cent level   Critical Absolute Values: 5%: 0.138

**Significant at the 99 per cent level   1%: 0.181

S.L.'s/1000:   Small Lymphocytes per 1000 blood cells   L.L.'s/1000:   Large Lymphocytes per 1000 blood cells

% S.L.'s: Small Lymphocytes per 100 Leucocytes   % L.L.'s: Large Lymphocytes per 100 Leucocytes

Temperature = Water Temperature at time of Autopsy

correlated with interrenal nuclear diameters or No. Nu.'s. However, numbers of neutrophils per hundred leucocytes (% Nt.'s) were positively correlated with interrenal nuclear diameters, and negatively correlated with numbers of interrenal nuclei per defined area. Numbers of immature erythcocytes per thousand blood cells were positively correlated with I.N.D.'s, although not significantly correlated with No. Nu.'s.

Epsilon cell nuclear diameters (E.N.D.'s) were positively correlated with lengths and weights. E.N.D.'s were very significantly positively correlated with interrenal cell nuclear diameters, while being very significantly negatively correlated with numbers of interrenal nuclei per defined area of interrenal tissue. Both S.L.'s/1000 and % S.L.'s were negatively correlated with epsilon cell nuclear diameters. L.L.'s/1000 and % L.L.'s were not significantly correlated with E.N.D.'s, whereas Nt.'s/1000 and % Nt.'s were positively correlated with E.N.D.'s.

Both numbers of small lymphocytes per thousand blood cells and numbers of small lymphocytes per hundred leucocytes were positively correlated with thrombocyte counts from all experiments. S.L.'s/1000 and % S.L.'s were also positively correlated, as were Nt.'s/1000 and % Nt.'s. However, L.L.'s/1000 and % L.L.'s were not significantly correlated.

Values for hematocrits from all experiments were positively correlated with S.L.'s/1000 and % S.L.'s, and negatively correlated with % Nt.'s. Hematocrits were also negatively correlated with interrenal nuclear diameters and numbers of immature erythrocytes.

Some very interesting correlations were found when variables measured from fish were compared with water temperatures at the time of autopsy. Both interrenal cell nuclear diameters and epsilon cell nuclear diameters were very significantly negatively correlated with temperatures.

Numbers of interrenal cell nuclei per defined area, on the other hand,
were positively correlated with water temperatures. Temperatures were also
positively correlated with numbers of small lymphocytes per thousand blood
cells, numbers of small lymphocytes per hundred leucocytes and with
thrombocyte counts. Numbers of large lymphocytes per thousand blood cells
were positively (although not significantly) correlated with temperatures,
whereas numbers of neutrophils per thousand blood cells were significantly
negatively correlated with temperatures. Numbers of immature erythrocytes
were negatively correlated with temperatures. Finally, hematocrits were
positively correlated with water temperatures at the time of autopsy.

DISCUSSION

Many investigators have noticed an effect in higher vertebrates of duration of exposure to varying periods of light and darkness on their endocrine functions. The single environmental factor generally believed to be most critical in regulating the development and periodicity of reproductive activity is light (Gorbman and Bern, 1962). Experimental manipulation of daily photoperiod affects the release of pituitary gonadotropin in rats (Negro-Vilar et al., 1968). Several studies have reported circadian rhythms in adrenocortical secretion in mammals (Guilleman et al., 1959; Mills, 1966). These rhythms are altered by exposure to constant light (Halberg et al., 1953; Cheifetz et al., 1968).

In teleost fishes, experimental manipulation of daily photoperiod causes behavioral changes (Northcote, 1958; Baggerman, 1960b; McCrimmon and Kwain, 1966; Chaston, 1968). Photoperiod affects thyroid activity (Eales, 1965) and the degree of silvering (Hoar, 1965; Johnston and Eales, 1968) of salmonids undergoing the transformation from parr to smolt. Gonadal maturation and reproductive activities of the stickleback are dependent on the photoperiod (Baggerman, 1957; Hoar, 1962) as is prolactin secretion (Lam, 1965). Thus it appears that in teleosts, as in higher vertebrates, the activity of several endocrine organs is regulated or modified through the changing length of the photoperiod throughout the season.

Only one previous report has appeared on the effect of a marked change in daily photoperiod on the interrenal activity of a teleost fish. Rasquin and Rosenbloom (1954) submitted Astyanax mexicanus to prolonged periods of continuous darkness. Hypertrophy and hyperplasia of the

interrenal tissue was observed after two weeks of darkness, accompanied by a loss of lymphoid elements from the head kidney, thymus and spleen. This stimulation of the interrenal tissue, as well as other endocrinological alterations, was observed in fish maintained in darkness for several months. Rasquin and Rosenbloom considered raising fish in darkness as equivalent to subjecting them to long-continued stress.

According to criteria used in the present study to assess interrenal activity (degree of hyperplasia, nuclear and cell size, nucleolar prominence, appearance of cytoplasm, frequency of mitotic figures, degree of vascularization of head kidney), the interrenal tissue of fish maintained in continuous darkness for varying numbers of days was generally more active than that of corresponding controls subjected to a twelve hour photoperiod. In addition, mean small-lymphocyte and thrombocyte counts for darkness-maintained fish were generally lower than values for corresponding control samples. Thus, as in the study by Rasquin and Rosenbloom (1954), exposure of fish to continuous darkness stimulated the interrenal tissue of juvenile coho salmon. This stimulation was reflected in characteristic alterations of the numbers of circulating leucocytes. However, unlike the previous study, the initial increased activity of the interrenal tissue of juvenile coho salmon exposed to continuous total darkness was not maintained with prolonged periods of darkness. The interrenal tissue of both dark-maintained and corresponding control fish appeared to be initially stimulated following transfer of fish from stock tanks to individual compartments, as evidenced by the marked differences in interrenal tissue when these samples and the stock-tank sample were compared. Additionally, mean nuclear diameters of epsilon cells in the rostral pars distalis of the initial control and experimental samples were very significantly larger than the mean epsilon

nuclear diameter of the stock-tank sample. However, interrenal activity of both experimental fish and of corresponding controls decreased with increasing numbers of days that fish were maintained in compartments prior to sacrifice. Thus, evidence is provided for an involvement of the pituitary-interrenal axis of juvenile coho salmon during the acclimatization of these fish to an altered environment. A similar difference in activity of the pituitary-interrenal axis was observed when the uninjected control sample of fish maintained in compartments for two weeks was compared with the stock-tank sample in the ACTH-injection experiment. Chavin's (1964) observation of increased blood glucose levels in goldfish transferred from one aquarium to another further supports the present evidence for a participation of the pituitary-interrenal axis of fish in the acclimatory response to environmental alterations. Exposure of fish to continuous darkness in the present study could be considered an additional environmental alteration, requiring further interrenal activity for the fish to acclimatize to its surroundings.

Examination of gut contents on autopsy revealed no decrease in amount of food consumed by dark-maintained fish compared with corresponding controls. Therefore the increased interrenal activity observed in dark-maintained fish cannot be attributed to starvation. Likewise, it is unlikely that this difference is due to a possible diurnal variation in secretory activity of the interrenal tissue, since all fish were sacrificed during the dark phase of the daily photoperiod. It has been observed by myself and by Hoar (1951) that coho fry in their natural habitat remain quiescent at night. Unfortunately, the activity of coho fry maintained in continuous total darkness for varying numbers of days is unknown.

Unlike the continuous-darkness experiments, no consistent

differences in activity of the interrenal tissue of juvenile coho salmon were found when fish maintained in continuous light for varying numbers of days and corresponding control fish receiving a twelve hour photoperiod were compared. Furthermore, the interrenal tissue of fish maintained in continuous light for 2, 4 or 8 days was very active according to criteria used to assess interrenal activity in these studies. Therefore, exposure of fish to prolonged periods of continuous light does not inhibit increased activity of the pituitary-interrenal axis in this study. This observation tends to negate the possibility that the inactivity of the interrenal tissue found in Bertrand Creek samples of coho during the late spring, summer and early fall was due to the inhibitory effect of a long photoperiod.

The interrenal tissue of juvenile coho salmon exposed to continuously flashing lights for varying numbers of days was generally more active according to criteria used in this study than was the interrenal tissue of corresponding control fish receiving a twelve hour photoperiod. In addition, both small-lymphocyte and thrombocyte counts were lower for fish exposed to varying periods of flashing light than for corresponding controls. Furthermore, interrenal activity of both flash-exposed fish and corresponding controls decreased with increasing numbers of days that fish were maintained in individual compartments prior to sacrifice. This decreased interrenal activity with time was accompanied by a general increase in the small-lymphocyte count. From these observations it appears that a continuously flashing light is an abnormal environmental stimulus to juvenile coho salmon, evoking an increased activity of the interrenal tissue with concomitant alterations in numbers of circulating leucocytes. However, the decreased interrenal activity of both flash-exposed fish and corresponding controls with increasing numbers of days of residence in compartments might

represent an acclimatization of these fish to an altered environment, similar to that discussed in the continuous-darkness experiments.

It was noted in the flashing light experiments that hematocrits were consistently (although not significantly) lower for fish exposed to varying periods of flashing lights than corresponding control values. Randall et al. (1967) reported that during moderate exercise in trout the oxygen transfer factor increased almost fivefold, and suggested that factors augmenting gas exchange at the gills during exercise should also augment water exchange. An uptake of water during exercise of trout has also been reported (Stevens, 1968). Although variations in activity of flash-exposed fish compared with controls were not observed in the present study, it is possible that exposure of juvenile coho salmon to a continuously flashing light caused an increase in activity, resulting in a slight but consistent decrease in hematocrit.

Several reports have appeared on variations in the adrenocortical function of mammals exposed to environmental temperature extremes. Kotby et al. (1967) found a highly significant elevation of plasma corticosterone levels in rats exposed to a high ambient temperature. The adrenal cortex of mammals also is activated during cold acclimation (Heroux and Hart, 1954; Heroux, 1960). However, increasing evidence indicates that hyperactivity of the adrenal cortex is essential only during the initial acclimatory response to cold (Boulouard, 1963; Straw and Fregly, 1967). It is speculated that the enhanced adrenal-pituitary function is related to the increased metabolic demands for heat production which may be met initially by an increased utilization of body protein, under the influence of adrenocortical hormones, but later by an increased caloric intake.

The only published report of the response of the adrenocortical

(interrenal) tissue of teleost fishes to temperature extremes is that of
Mahon et al. (1962). Goldfish were exposed to high and low lethal
temperatures, and the histology of the interrenal tissue examined. High
temperature produced an exhaustion of large areas of the interrenal cells,
while chilling temperatures caused a mild stimulation of the interrenal
tissue. Unfortunately, since temperature extremes used in this study were
lethal, no evidence was provided as to the participation of the pituitary-
interrenal axis of fishes during temperature acclimation.

In Section II, an increased activity of the interrenal tissue was
reported for juvenile coho salmon taken from Bertrand Creek during the
winter. This increased interrenal activity was tentatively attributed to
the decreased ambient water temperature, although other seasonal alterations
which might account for this variation in activity were considered. In this
Section, considerable evidence is provided which indicates that the activity
of the interrenal tissue of juvenile coho salmon is increased by exposure
of these fish to cold water temperatures in the laboratory, and that this
increased interrenal activity is reflected in characteristic alterations
in numbers of circulating leucocytes. In the continuous-darkness
experiments, a marked lymphopenia and thrombocytopenia was observed in fish
from the eight day experiment (water temp. 5°C.) compared with other
samples obtained when ambient temperatures were warmer. The interrenal tissue
of these fish was very active according to histological criteria. A
comparison of stock-tank samples in both the continuous-light experiments
and the flashing-light experiments provides further evidence for an
inverse relationship between ambient water temperatures at the time of
autopsy and the activity of the interrenal tissue. In addition, in the
continuous-light experiments, the interrenal tissue of both experimental and

control samples of fish maintained in compartments for considerable periods of time was more active than that of previous samples. This variation in interrenal activity, along with characteristic alterations in small-lymphocyte counts, could be attributed to a sharp decrease in water temperatures in the latter experiments. Contrary to this, water temperatures rose in the latter flashing-light experiments. Correspondingly, the activity of the interrenal tissue of both flash-exposed fish and controls decreased with increasing water temperatures, while mean small-lymphocyte counts increased.

Further support for the hypothesis that there is an interrelationship between water temperature and interrenal activity is provided by correlation coefficients. In both the continuous-light and flashing-light experiments, ambient water temperatures at the time of autopsy were very significantly negatively correlated with nuclear diameters of interrenal cells and with estimates of interrenal cell size, and were positively correlated with small-lymphocyte counts. Thus, it appears from these studies that the pituitary-interrenal axis of juvenile coho salmon is stimulated by exposure of fish to low ambient water temperatures, and that this stimulation is accompanied by a characteristic lymphopenia. These findings are consistent with Fry and Hochachka's (1970) model, proposing active participation of glucocorticoids in teleost fishes during cold acclimation. Since temperatures along with other environmental variables fluctuated markedly throughout these studies, it is impossible in this investigation to ascertain whether or not the increased activity of the interrenal tissue is essential only during the initial acclimatory response to cold.

In Section I, it was found that variations in the activity of the pituitary-interrenal axis of coho fry are reflected in characteristic changes

in the numbers of circulating leucocytes. Similar alterations in circulating leucocyte counts associated with increased interrenal activity were found in juvenile coho salmon exposed to low ambient water temperatures in their natural habitat or in the laboratory. However, these studies do not establish that the alterations in leucocyte counts with temperature are in reality a reflection of the activity of the pituitary-interrenal axis, rather than a direct effect of temperature on the hematopoietic tissue or on the circulating leucocytes. To clarify this relationship, it would be worthwhile to expose both hypophysectomized and sham-hypophysectomized fish (or fish injected with metopirone, an inhibitor of adrenocorticosteroid synthesis) to temperature extremes, and to determine the effect on counts of circulating leucocytes.

A marked seasonal difference in thermal resistance has been reported in studies of goldfish. Fish were more resistant to heat during the summer and more resistant to cold in the winter (Hoar, 1955). This difference in thermal resistance is attributed to seasonal variations in photoperiod, since long photoperiods - like summer conditions - increase resistance to heat while short photoperiods - like winter conditions - increase resistance to cold temperature (Hoar, 1956; Hoar and Robertson, 1959). Thus seasonally changing photoperiods adjust the metabolism of the fish to anticipate abrupt seasonal temperature changes. In the present study it has been concluded that the pituitary-interrenal axis of juvenile coho salmon is activated during cold temperature acclimation. Furthermore, evidence is presented which indicates that the interrenal tissue is more active when these fish are maintained in the absence of light, as compared with interrenal tissue of corresponding controls receiving a twelve hour photoperiod. From these findings, it is possible to speculate that adjustments in the activity

of the pituitary-interrenal axis of fish are involved in the temperature

compensations that depend on photoperiod. Decreasing photoperiods in the

autumn possibly might activate the pituitary-interrenal axis of fish in

order to facilitate the metabolic adjustments observed in teleost fishes

during cold temperature acclimation. Thus the metabolism of the fish would

be adjusted to anticipate an abrupt decline in temperature, which might

otherwise be lethal. This hypothesis is speculative, and requires a careful

examination of the activity of the pituitary-interrenal axis of fish

maintained under differing controlled conditions of photoperiod and

temperature for confirmation or rejection.

Correlation coefficients were determined for variables measured in

juvenile coho salmon involved in all experiments (excluding injection

experiments). These correlations were based on data measured in 270 fish.

It was felt that this large sample size would make it possible to clarify the

interrelationships of the variables measured.

The very significant negative correlation between I.N.D.'s and

No. Nu.'s establishes that the nuclear hypertrophy is accompanied by a

corresponding increase in cell size. The very significant positive correlation

between I.N.D.'s and E.N.D.'s and the very significant negative correlation

between E.N.D.'s and No. Nu.'s are consistent with the hypothesis that the

epsilon cells are the ACTH-producing cells of juvenile coho salmon. These

correlations also indicate that variations in nuclear diameters of epsilon

cells reflect alterations in activity of these cells.

Several significant correlations were found between differential

leucocyte counts and the variables measured to assess the activity of the

interrenal cells and epsilon cells. Both S.L.'s/1000 and % S.L.'s were

very significantly negatively correlated with I.N.D.'s and E.N.D.'s, while

small-lymphocyte counts were very significantly positively correlated with No. Nu.'s. It is concluded from these correlations and from other data previously discussed that a decrease in numbers of circulating small lymphocytes in juvenile coho salmon reflects an increase in activity of the pituitary-interrenal axis. On the other hand, thrombocyte counts were not significantly correlated with I.N.D.'s, No. Nu.'s or E.N.D.'s, although numbers of circulating thrombocytes were very significantly positively correlated with small-lymphocyte counts. Since injections of ACTH, cortisol acetate or dexamethasone caused a thrombocytopenia, it is thought that the number of circulating thrombocytes is related to the activity of the pituitary-interrenal axis of juvenile coho salmon; the absence of significant correlations found here might reflect a difference in sensitivity of these cells to endogenous plasma cortisol levels compared with the sensitivity of small lymphocytes.

In the various experiments, no obvious differences due to environmental alterations in the 'absolute' numbers of circulating neutrophils was detected. However, this apparent lack of variation in neutrophil counts could be a consequence of the extremely low numbers of neutrophils relative to the thousand blood cells counted in each smear. When correlations were determined for variables measured from fish from all experiments, Nt.'s/1000 were significantly positively correlated with E.N.D.'s. Furthermore, Nt.'s/1000 and % Nt.'s were very significantly positively correlated. Therefore, as in mammals, a neutrophilia following increased activity of the pituitary-adrenocortical axis might conceivably occur in teleost fishes. However, this response, if present, is not readily detected by the methods employed in this study.

The many significant correlations between water temperature at the

time of autopsy and variables measured to assess activity of the interrenal

and epsilon cells, as well as between temperature and differential leucocyte

counts, again emphasize the negative correlation between this variable and

the activity of the pituitary-interrenal axis.

From the foregoing experiments, it is apparent that the pituitary-

interrenal axis of juvenile coho salmon is capable of marked fluctuations

in activity under laboratory conditions. It is noteworthy that the interrenal

tissue of fish transported from their natural environment and maintained

under varying environmental conditions in the laboratory is often far more

active according to the criteria used in these studies than the interrenal

tissue of fish sampled seasonally from Bertrand Creek. Small-lymphocyte and

thrombocyte counts also are often much lower for laboratory-maintained fish

than values from stream samples of juvenile coho salmon. It is difficult

to attribute a specific environmental variable to these differences, since

under laboratory conditions the fish are exposed to a variety of stimuli -

including sound, vibrations, unnatural lighting conditions, marked temperature

fluctuations and abnormal surroundings - which are not experienced by fish

in their natural habitat. Probably the increased interrenal activity

observed in juvenile coho salmon under laboratory conditions is induced by

numerous stimuli acting simultaneously, and is thus non-specific.

In conclusion, these studies aptly illustrate the necessity for

carefully standardized laboratory conditions if one wishes to study the

activity of the pituitary-interrenal axis of fish. In fact, since variations

in activity of one endocrine organ often affect the function of other

endocrine systems, a carefully controlled environment should be a

prerequisite to all endocrinological investigations with fishes. Furthermore,

fish transferred from their natural environment to the laboratory, or from

one environment to another within the laboratory, should be allowed a
considerable period of time for acclimatization to the altered environment
prior to experimentation.

In the present investigations, individual variations in differential
leucocyte counts were so great that there is a considerable degree of
statistical uncertainty as to the significance of these variations. Also,
measurements within a sample of nuclear diameters of interrenal cells varied
considerably from cluster to cluster and from fish to fish. Therefore,
further studies utilizing these criteria for assessing the activity of the
pituitary-interrenal axis of fishes should employ a larger sample size.
The development of a microtechnique for determining cortisol levels in
microliter quantities of plasma would greatly assist studies of this sort.

SUMMARIES

SECTION I

1. Injections of ACTH resulted in a stimulation of the interrenal tissue of coho salmon fry. The stimulated tissue was characterized by a marked hypertrophy of the interrenal cells, by very prominent nucleoli, by increased nucleolar and cytoplasmic basophilia (RNA), by increased numbers of mitotic figures, by increased vascularization of the head kidney, and by hyperplasia of the interrenal tissue.

2. ACTH caused a dosage-related increase in mean diameters of interrenal cell nuclei and in mean cell size. Measurements of interrenal nuclear diameters were very significantly negatively correlated with numbers of interrenal nuclei per defined area of interrenal tissue.

3. Injections of both cortisol acetate and dexamethasone caused a marked atrophy of interrenal cells, characterized by a decrease in interrenal nuclear diameters and cell sizes, by irregularly shaped nuclei, by an unevenly stained, vacuolated cytoplasm, by intercellular spaces within clusters of interrenal cells, and by an absence of mitotic figures. Neither cortisol nor dexamethasone increased the vascularization of the head kidney.

4. All dosages of ACTH and cortisol acetate caused a decrease in the mean nuclear diameters of epsilon cells in the rostral pars distalis. In addition, a degranulation of epsilon cells following ACTH-, cortisol acetate- or dexamethasone-injections was observed. Dexamethasone-injections did not decrease nuclear diameters of epsilon cells significantly.

5. Pycnosis and shrinkage of lymphocytes within the hematopoietic tissue of the head kidney occurred following injections of either ACTH, cortisol acetate or dexamethasone. Numerous spaces or vacuolations were observed in the

hematopoietic tissue.

6. A very marked decrease in circulating small lymphocytes following administration of all dosages of ACTH, cortisol acetate and dexamethasone was found.

7. A thrombocytopenia resulted from ACTH-, cortisol acetate- or dexamethasone-administration.

8. Numbers of circulating neutrophils were unaffected by cortisol or dexamethasone; however the maximal dosage of ACTH caused a definite neutrophilia.

9. No histological or histometric differences in the epsilon cells, inter-renal tissue or head kidney tissue were found between saline-injected and non-injected controls in either the ACTH- or cortisol-injection experiments. In addition, differential blood cell counts were similar.

10. It is concluded that the interrenal tissue of coho fry is capable of marked variations in activity, that this tissue is under pituitary control, and that a negative-feedback mechanism probably operates between the inter-renal and the pituitary gland. Additionally, changes in pituitary-adreno-cortical activity are reflected in characteristic alterations in numbers of specific circulating leucocytes.

SECTION II

1. According to the criteria used in this study to assess pituitary-adreno-cortical activity, the pituitary-interrenal axis of juvenile coho salmon in their natural habitat is inactive, from the time of emergence in spring, and through summer and early autumn, compared with the winter and spring samples of yearling coho. Hyperplasia of interrenal tissue, and increases in diameters of interrenal cell nuclei and epsilon cell nuclei, in interrenal cell size, in nucleolar prominence, in numbers of mitotic figures in

interrenal tissue and in the degree of vascularization of head kidney were found in the winter and spring samples of yearling coho compared with previous samples.

2. Numbers of circulating small lymphocytes were decreased in the winter and spring samples of yearling coho compared with summer and fall samples. Additionally, numbers of small lymphocytes were negatively correlated with nuclear diameters of interrenal cells and epsilon cells, and positively correlated with numbers of interrenal nuclei per defined area.

3. Thrombocyte counts were highest in summer samples and decreased in the winter sample, although counts rose again in the spring with increasing water temperatures. Numbers of circulating neutrophils were low in all samples, although highest for the winter sample.

4. Small-lymphocyte and thrombocyte counts were very low in the newly-emerged coho fry. It is suggested that this leucopenia is a developmental phenomenon rather than a reflection of increased activity of the pituitary-interrenal axis.

5. Water temperatures at the time of autopsy were negatively correlated with nuclear diameters of interrenal cells and epsilon cells, and were positively correlated with numbers of interrenal nuclei per defined area, with small lymphocyte counts and with thrombocyte counts. It is suggested that the increased activity of the pituitary-interrenal axis observed in the winter sample of juvenile coho salmon is related to cold-temperature acclimation.

6. The interrenal tissue of the later spring sample of yearling coho salmon was more active than that of earlier spring samples. This increased interrenal activity was associated with a silvering and loss of parr marks in these fish. It is suggested that the pituitary-interrenal axis of juvenile coho salmon is

activated during the transformation of fish from parr to smolt.

SECTION III

1. According to histological observations and histometric criteria, the interrenal tissue of juvenile coho salmon maintained in continuous darkness for varying numbers of days was more active than that of corresponding controls subjected to a twelve hour photoperiod. Additionally, small-lymphocyte and thrombocyte counts for darkness-maintained fish generally were lower than values for corresponding control samples.

2. The interrenal tissue and epsilon cells of both dark-maintained and corresponding control fish initially were stimulated following transfer of fish from stock-tanks to individual compartments. However, interrenal activity of both dark-maintained fish and of corresponding controls decreased with increasing numbers of days that fish were maintained in compartments prior to sacrifice.

3. No consistent differences in activity of the interrenal tissue were found when fish maintained in continuous light for varying numbers of days and corresponding control fish were compared.

4. Interrenal tissue of fish exposed to continuously flashing lights for varying numbers of days generally was more active than that of corresponding control fish. In addition, both small-lymphocyte and thrombocyte counts were lower for fish exposed to varying periods of flashing light than for corresponding controls.

5. Interrenal activity of both flash-exposed fish and corresponding controls decreased with increasing numbers of days that fish were maintained in compartments prior to sacrifice. Results from these experiments and from the continuous-darkness experiments suggest that the pituitary-interrenal axis

is involved in the acclimatization of juvenile coho salmon to an altered environment.

6. Considerable evidence in this Section indicates that the activity of the interrenal tissue of juvenile coho salmon is increased by exposure of these fish to cold water temperatures in the laboratory, and that this increased interrenal activity is accompanied by a characteristic lymphopenia.

7. In the various experiments, no obvious differences in the 'absolute' numbers of circulating neutrophils were detected following environmental changes.

8. When correlation coefficients were determined for variables measured in all experiments, nuclear diameters of epsilon cells were very significantly positively correlated with interrenal cell nuclear diameters and very significantly negatively correlated with numbers of interrenal cell nuclei per defined area. These correlations are consistent with the hypothesis that the epsilon cells are the ACTH-producing cells of these fish, and that variations due to environmental alterations in activity of the interrenal tissue are mediated by the pituitary gland.

9. According to correlation coefficients of variables measured in all experiments, small-lymphocyte counts were very significantly negatively correlated with nuclear diameters of interrenal cells and epsilon cells, and were very significantly positively correlated with numbers of interrenal nuclei per defined area of interrenal tissue. It is concluded from these correlations and from other data that a decrease in numbers of circulating small lymphocytes in juvenile coho salmon reflects an increase in activity of the pituitary-interrenal axis.

10. The pituitary-interrenal axis of juvenile coho salmon maintained under laboratory conditions is capable of marked fluctuations in activity as a

result of environmental alterations. Furthermore, the activity of this axis in laboratory-maintained fish is often far greater than that of fish in their natural habitat.

TABLE I - APPENDIX I

Leishman-Giemsa Staining Technique

Air-dry blood smears	6 - 12 hours
Flood smear with Leishman stain (stock sol'n., Harleco)	5 minutes
Add equal amount of Giemsa (3.5 ml. Allied Chem. stock sol'n. in 50 ml. of pH 6.4 phosphate buffer)	3 - 4 minutes
Rinse with pH 6.4 phosphate buffer	
Rinse in running tap water	
Air-dry slide in a vertical position	minimum 1 hour
Coverslip with Permount (Fisher)	

TABLE I - APPENDIX II

SUMMARY STATISTICS: ACTH-INJECTION EXPERIMENTS

VARIABLE	LOW	HIGH	AVERAGE	STD. DEV.	VARIANCE
Length (cm.)	6.4	8.5	7.6	0.56	0.32
Weight (gm.)	2.5	6.0	4.1	0.92	0.85
Hematocrit	8.5	32.5	22.2	6.19	38.35
S.L.'s/1000	0.0	53.0	13.1	16.50	272.26
% S.L.'s	27.0	99.0	65.7	24.38	594.44
L.L.'s/1000	0.0	1.0	0.1	0.31	0.09
% L.L.'s	0.0	33.0	10.3	9.15	83.78
Nt.'s/1000	0.0	10.0	2.6	3.20	10.26
% Nt.'s	1.0	53.0	22.4	16.27	264.76
Imm. R.B.C.'s/1000	51.0	443.0	180.0	98.77	9755.30
Thrombocytes	2.0	30.0	12.2	8.79	77.22
No.Nu	10.1	25.6	16.3	4.41	19.52
I.N.D. (microns)	5.84	7.60	6.68	0.48	0.23
E.N.D. (microns)	4.97	5.74	5.34	0.21	0.04

TABLE II - APPENDIX II

SUMMARY STATISTICS: CORTISOL-INJECTION EXPERIMENTS

VARIABLE	LOW	HIGH	AVERAGE	STD. DEV.	VARIANCE
Length (cm.)	7.0	9.1	8.0	0.51	0.26
Weight (gm.)	3.1	6.6	4.7	0.94	0.89
Hematocrit	16.0	33.5	25.1	3.90	15.23
S.L.'s/1000	0.0	23.0	6.0	8.09	65.37
% S.L.'s	14.0	97.0	63.7	23.92	572.34
L.L.'s/1000	0.0	0.0	0.0	0.00	0.00
% L.L.'s	0.0	20.0	6.2	6.67	44.56
Nt.'s/1000	0.0	6.0	0.9	1.37	1.88
% Nt's	3.0	65.0	29.7	18.58	345.29
Imm. R.B.C.'s/1000	6.0	233.0	115.2	75.17	5650.50
Thrombocytes	0.00	20.0	8.9	6.28	39.46
No.Nu.	17.7	30.5	25.6	4.31	18.59
I.N.D. (microns)	5.36	6.42	6.07	0.28	0.08
E.N.D. (microns)	4.88	5.77	5.30	0.24	0.06

TABLE III - APPENDIX II

SUMMARY STATISTICS: DEXAMETHASONE-INJECTION EXPERIMENTS

VARIABLE	LOW	HIGH	AVERAGE	STD. DEV.	VARIANCE
Length (cm.)	7.5	9.5	8.6	0.56	0.31
Weight (gm.)	3.7	7.8	5.7	1.24	1.55
Hematocrit	17.0	29.0	24.3	4.17	17.42
S.L.'s/1000	0.0	11.0	2.1	3.81	14.49
% S.L.'s	39.0	82.0	58.4	15.22	231.65
L.L.'s/1000	0.0	1.0	0.1	0.30	0.09
% L.L.'s	0.0	16.0	6.8	4.40	19.36
Nt.'s/1000	0.0	6.0	1.5	2.11	4.47
% Nt.'s	17.0	49.0	32.8	12.34	152.36
Imm. R.B.C.'s/1000	6.0	192.0	77.9	66.12	4371.80
Thrombocytes	0.0	23.0	10.5	7.23	52.27
No.Nu.	24.5	31.2	27.2	2.37	5.63
I.N.D. (microns)	5.94	6.34	6.16	0.18	0.02
E.N.D. (microns)	5.11	5.72	5.33	0.19	0.03

TABLE IV - APPENDIX II

SUMMARY STATISTICS: BERTRAND CREEK SAMPLES

VARIABLE	LOW	HIGH	AVERAGE	STD. DEV.	VARIANCE
Length (cm.)	4.4	11.5	8.3	1.62	2.62
Weight (gm.)	1.1	14.5	6.6	3.46	11.97
Temperature (°C.)	4.0	18.5	10.5	4.13	17.03
S.L.'s/1000	0.0	64.0	18.4	15.26	233.00
% S.L.'s	53.0	99.0	88.2	10.46	109.30
L.L.'s/1000	0.0	2.0	0.2	0.46	0.21
% L.L.'s	0.0	12.0	2.6	3.11	9.68
Nt.'s/1000	0.0	5.0	0.7	1.00	1.00
% Nt.'s	1.0	38.0	8.9	8.79	77.31
Imm. R.B.C.'s/1000	0.0	238.0	58.4	57.30	3283.30
Thrombocytes	0.0	35.0	9.4	8.54	72.88
No.Nu.	20.4	35.2	27.7	3.66	13.41
I.N.D. (microns)	5.52	6.61	6.10	0.30	0.09
E.N.D. (microns)	4.72	5.77	5.34	0.29	0.09

TABLE V - APPENDIX II

SUMMARY STATISTICS: CONTINUOUS-DARKNESS EXPERIMENTS

VARIABLE	LOW	HIGH	AVERAGE	STD. DEV.	VARIANCE
Length (cm.)	5.6	9.9	7.8	0.98	0.97
Weight (gm.)	1.3	9.1	4.7	1.68	2.84
Hematocrit	20.0	45.0	32.1	5.96	35.54
Temperature (°C.)	5.0	13.0	9.0	2.14	4.57
S.L.'s/1000	0.0	52.0	17.4	11.17	124.80
% S.L.'s	53.0	100.0	88.6	10.45	109.13
L.L.'s/1000	0.0	3.0	0.3	0.57	0.32
% L.L.'s	0.0	12.0	2.2	2.77	7.67
Nt.'s/1000	0.0	5.0	0.7	0.92	0.84
% Nt.'s	0.0	35.0	8.2	7.68	59.03
Imm. R.B.C.'s/1000	2.0	183.0	49.7	30.98	960.00
Thrombocytes	0.0	49.0	13.7	9.55	91.24
No.Nu.	15.9	30.5	24.4	2.74	7.54
I.N.D. (microns)	5.96	7.08	6.48	0.20	0.04

TABLE VI - APPENDIX II

SUMMARY STATISTICS: CONTINUOUS-LIGHT EXPERIMENTS

VARIABLE	LOW	HIGH	AVERAGE	STD. DEV.	VARIANCE
Length (cm.)	7.8	10.0	8.7	0.51	0.26
Weight (gm.)	4.3	8.9	6.3	1.07	1.14
Hematocrit	13.5	29.0	22.7	2.91	8.48
Temperature (°C.)	4.0	9.5	6.4	2.11	4.45
S.L.'s/1000	0.0	31.0	6.5	6.19	38.34
% S.L.'s	39.0	98.0	76.6	13.66	186.54
L.L.'s/1000	0.0	1.0	0.1	0.29	0.08
% L.L.'s	0.0	10.0	2.3	2.27	5.17
Nt.'s/1000	0.0	6.0	1.3	1.35	1.83
% Nt.'s	2.0	51.0	21.0	12.08	145.95
Imm. R.B.C.'s/1000	0.0	227.0	69.4	39.87	1589.70
Thrombocytes	0.0	25.0	8.2	6.69	44.74
No.Nu.	18.1	29.5	24.2	2.34	5.49
I.N.D. (microns)	5.95	7.11	6.58	0.23	0.05

TABLE VII - APPENDIX II

SUMMARY STATISTICS: FLASHING-LIGHT EXPERIMENTS

VARIABLE	LOW	HIGH	AVERAGE	STD. DEV.	VARIANCE
Length (cm.)	8.0	11.8	9.3	0.62	0.39
Weight (gm.)	4.8	16.0	7.3	1.78	3.18
Hematocrit	11.5	32.5	22.6	4.30	18.52
Temperature (°C.)	5.0	9.5	7.3	1.49	2.21
S.L.'s/1000	0.0	26.0	6.8	5.05	25.48
% S.L.'s	43.0	98.0	81.6	11.48	131.75
L.L.'s/1000	0.0	1.0	0.1	0.31	0.09
% L.L.'s	0.0	20.0	1.5	2.74	7.51
Nt.'s/1000	0.0	4.0	1.0	1.10	1.21
% Nt.'s	2.0	47.0	17.0	10.26	105.20
Imm. R.B.C.'s/1000	0.0	94.0	38.2	23.67	560.40
Thrombocytes	0.0	27.0	10.2	6.16	37.88
No.Nu.	17.4	31.4	23.2	3.16	10.01
I.N.D. (microns)	5.59	6.89	6.47	0.26	0.07

TABLE VIII - APPENDIX II

SUMMARY STATISTICS: ALL EXPERIMENTS

VARIABLE	LOW	HIGH	AVERAGE	STD. DEV.	VARIANCE
Length (cm.)	4.4	11.8	8.5	1.06	1.12
Weight (gm.)	1.1	16.0	6.2	2.19	4.80
Hematocrit	11.5	45.0	25.3	6.05	36.59
Temperature (°C.)	4.0	18.5	8.6	2.84	8.05
S.L.'s/1000	0.0	55.0	11.5	10.33	106.67
% S.L.'s	39.0	100.0	83.8	12.47	155.42
L.L.'s/1000	0.0	3.0	0.2	0.43	0.18
% L.L.'s	0.0	20.0	1.8	2.41	5.81
Nt.'s/1000	0.0	6.0	0.9	1.08	1.18
% Nt's	0.0	51.0	14.3	11.37	129.37
Imm. R.B.C.'s/1000	0.0	238.0	52.2	44.99	2023.70
Thrombocytes	0.0	49.0	10.6	7.83	61.34
No.Nu.	15.9	35.2	24.7	3.28	10.77
I.N.D. (microns)	5.35	7.11	6.42	0.30	0.09
E.N.D. (microns)	4.72	6.09	5.46	0.30	0.09

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