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# *ENDOCRINOLOGICAL AND HEMATOLOGICAL STUDIES IN FUNDULUS HETEROCLITUS (LINN.)*

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# Endocrinological and Hematological Studies in *Fundulus heteroclitus* (Linn.)

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# *Endocrinological and Hematological Studies in Fundulus heteroclitus (Linn.)*[1]

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## TABLE OF CONTENTS



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See p. 55 for Russian Abstract.

## ABSTRACT

The following blood cell types occur in the killifish, *Fundulus heteroclitus* (Linn.): erythrocytes, lymphocytes, eosinophils and thrombocytes; basophils and neutrophils are lacking. During the breeding season hemoglobin and red cell counts are higher in males than in females; during sexual regression the red cell count of females increases and that of males decreases to a common intermediate value. The hemoglobin and red cell count were higher in laboratory-kept fish (20° C) than in those living in a natural environment. In fish kept several months in fresh water, the hemoglobin and red cell count were higher than in those kept in salt water; there was in the former a mild lymphocytosis but a marked decrease in the number of circulating eosinophils. Hypothyroidism resulting from radioactive iodine had little or no effect on the blood picture. Pancytopenia, which developed after hypophysectomy, could be alleviated (at least in males) by chronic administration of ACTH, methyl-testosterone, or thyrotropic hormone (containing luteinizing hormone); prolactin was less effective and intermedin had no beneficial action. Changes in hemoglobin and red cell counts resulting from saline injections, cold shock, cortisol or ACTH gave conflicting results. Cold shock revealed a characteristic pattern of response in respect to the abundance of leucocytes in intact fish: lymphopenia after one hour, lymphocytosis after two hours. Results of other treatments can be interpreted in terms of this diphasic reaction; mild shock (saline injection) tended to elicit the first phase, high doses of ACTH caused lymphocytosis, and cortisol had either the one or the other effect depending on the state of maturation (lymphopenia during sexual regression changing to lymphocytosis with the approach to sexual maturation). Only the second phase of the response could be elicited after hypophysectomy. Changes in the number of eosinophils tended to follow the response of the lymphocytes. Eosinophils disappeared from the blood stream in fish which had been hypophysectomized for more than two months; saline injection, ACTH, or cortisol caused their transitory reappearance. Permanent restoration was associated with the improved hematological picture resulting from chronic treatment with ACTH or prolactin, but intermedin inhibited the response to the latter hormone.


## INTRODUCTION

Fish have been used for experimental purposes since the end of the 18th century (probably earlier) and have served as excellent tools for many investigations (Nigrelli, 1953; Pickford, 1953). A survey of the literature reveals that research in fish hematology has been done mostly in the fields of experimental and developmental biology, such as physiology, embryology, and genetics; relatively few reports have been devoted to the normal morphology of the blood elements.

Although Gulliver's classical work on blood morphology appeared in 1845, it was not until 1899 and 1900 that Rawitz described the elements in the circulating blood of fishes. Between 1904 and 1911, Anna Drzewina published a series of articles on fish hematology and Hoffmeyer (1907) produced his excellent study of normal and abnormal fish blood. A dearth in hematological research ensued until 1927, when Schlicher gave a detailed report on blood counts, hemoglobin values, and erythrocyte sizes in a large number of fishes. Then, almost a century after the first comprehensive work on fish hematology, two excellent reviews appeared; the first by Grodzinski (1938) and the second

by Kalashnikov (1939). So far as can be determined, no other extensive hematological studies were published until 1947, when Yokoyama conducted a thorough investigation of the perch blood. This was followed in 1949 by a study on the hematology of the silver salmon by Katz and by studies on the rainbow trout by Weinreb (1955). In 1956 Jakowska provided an excellent review of the morphology and nomenclature of blood cells in teleosts.

The species used by the workers mentioned in the preceding paragraph are relatively large and are not easily maintained in research laboratories that lack running water. But *Fundulus heteroclitus*, not much longer than five inches when sexually mature, is small enough so that an adequate number can be kept in standing water under laboratory conditions. Furthermore, this fish, being euryhaline, can be studied in both salt and fresh water. Also, it is one of the most popular native species used for research in the United States. However, no hematological data are available for this or other representatives of the same genus. Because of this signal lack, a thorough hematological study of this killifish is needed. Furthermore, such data should provide a valuable background for work dealing with endocrinology and nutrition.

It is known that in man and other mammalian species the erythrocyte count of an adult male is normally higher than that of a female (Scarborough, 1930–1931), a difference that has been attributed to the influence of sex hormones (Gordon and Charipper, 1947). During the New England winter, *F. heteroclitus* goes into complete sexual regression, and if the number of red blood cells in fish, as in mammals, is under the influence of sex hormones, then one might expect to find little difference in red blood cell counts of male and female killifish during this period of hormonal inactivity. It is therefore necessary to investigate not only sexual but also seasonal differences.

In many instances fish are used for experimental purposes almost immediately or within a few days after capture. Thus one may question whether hematological data gathered under such conditions are different from those obtained when fish are maintained for several weeks or even months under laboratory conditions. Furthermore, sea water aquaria are not commonly employed in inland laboratories, and many investigations have been made of euryhaline species in fresh rather than in salt water. Again one may question whether experimental data on fish kept in salt or fresh water can be compared indiscriminately. Parallel experiments should provide answers to these questions.

During winter regression, *F. heteroclitus* is in a state of partial hypopituitarism. Therefore a study of hypophysectomized fish might throw some light on the role of endocrines in regulating seasonal changes in the blood picture. Although effects of hypophysectomy on the blood of mammals have been described extensively (Gordon, 1954; Crafts, 1949), the effects on the hematology of fish are little known. A few reports indicate that in killifish, as in mammals, hypophysectomy results in a definite state of anemia (Pickford, 1953a, b; 1954a, b), but detailed studies are not available. Laur (1950) in-

ferred that no change in the blood picture was evident in hypophysectomized eels. It is of interest, therefore, to conduct a thorough hematological study on the anemia of hypophysectomized *F. heteroclitus* and to ascertain whether selected pituitary hormones would alleviate this condition, as they do in mammals (Li, *et al.*, 1957).

Particular interest centers on the role of adrenocorticotropin and the adrenal steroids. A general trend, reflected in the literature, seems to indicate that ACTH will increase the erythrocyte count of anemic mammalian recipients up to normal levels, but no effect on normal counts has been noted (Armour Laboratories, 1950). In man (Dougherty and White, 1943; Hill, *et al.*, 1948) and in other mammals (Reinhardt, *et al.*, 1944), ACTH depresses the white blood cell count. On the other hand an increase in the number of circulating white blood cells was observed when ACTH was administered to the fish *Tilapia macrocephala* (Slicher, 1951). Since it is not known what effect ACTH might have on red and white cell counts in normal or hypophysectomized killifish, this problem has also been investigated. Collateral to this study, the role of cortical steroids and cold shock is pertinent.

In view of the fact that thyroid function is known to influence the blood cell picture in man and laboratory animals (Gordon and Charipper, 1947), some experiments were made with thyrotropin (TSH) to ascertain the blood values of these fish and of hypothyroid killifish.

Because of seasonal changes and sex differences, the influence of methyltestosterone, a powerful androgen in fishes (Pickford and Atz, 1957), was studied. The influence of prolactin and intermedin was also investigated. An unsatisfactory pilot experiment with growth hormone has been excluded from this report.

## ACKNOWLEDGMENTS

I wish to express my deep appreciation to Professor Harry A. Charipper for his sponsorship of this work and for his helpful criticism and encouragement and to Dr. Grace E. Pickford for her constant help and suggestions. Facilities at Yale University were made possible through the co-operation of Dr. Daniel Merriman, Director of the Bingham Oceanographic Laboratory. Mrs. Emily E. Robertson and Mr. Robert A. Pawlikowsky assisted in the care of the fish and aided during autopsies. I am especially grateful to Miss Patricia J. Harris, who allowed me to collect blood from hypothyroid radiation-thyroidectomized fish, and to Mr. B. Kosto, who permitted me to use the blood from his prolactin-ACTH melanogenesis experiment. Oxygen determinations on the aquarium water were made by Dr. Gordon A. Riley of the Bingham Oceanographic Laboratory. Last but not least I express my sincere thanks to Dr. Ross F. Nigrelli, who stimulated my interest in fish hematology and who gave much help and constructive criticism.

## MATERIALS AND METHODS

The fish used in these experiments were caught occasionally with a hand net and more commonly with a trap at a small inlet of Long Island Sound near New Haven, Connecticut. They were transported in buckets of brackish water to the laboratory, where the water was vigorously aerated while the fish were being sorted and inspected for distribution into experimental tanks of various sizes. Large tanks containing about 40 gallons of water were used for reserve specimens, 20 gallon tanks containing 16 gallons of standing sea water were employed for long-term injection experiments, and 10 gallon tanks holding 8 gallons of either salt water or dechlorinated tap water were used for parallel experiments in these two media.

Salt water, obtained from Milford, Connecticut, was transported in glass carboys to the laboratory by truck. The fresh water system used has been described by Burden (1956). The pH of the fresh water was *c* 7.8, that of the salt water *c* 8.2; the latter had a salinity of *c* 25‰. The oxygen content of the fresh water was 6.09 ml/l (98% saturation), that of the salt water 5.08 ml/l (95% saturation). Marble chips were placed in all aquaria.

In general, not more than one fish was kept per two gallons of salt water; thus 6 to 8 fish could be held in 16 gallons and 4 to 5 in 8 gallons without changing the water. However, when more than one fish per two gallons of water were kept in a tank, the water had to be changed, usually at weekly intervals. The salinity, maintained at *c* 25‰, was checked periodically with a hydrometer and tap water was added about twice a week to compensate for loss by evaporation. The running fresh water system permitted more fish to be kept in these tanks (6 to 7 fish in 8 gallons of water) than in the salt water tanks.

The same environmental conditions were maintained for all aquarium-kept fish: a temperature between 19° and 21°C and daily illumination of eight hours unless otherwise stated. They were provided with a daily diet of Aronson's mixture, containing a trace of iodine (Pickford, 1953a); this was supplemented with frozen brine shrimp two or three times a week. The tanks were cleaned daily. Approximately once a month the gills of each fish were inspected for copepods, which, if present, were removed with fine forceps. Fish were marked either with colored beads (Pickford, 1953a) or, more frequently, with fin clips.

Two different procedures were employed in studies of the response to experimental injections. In the so-called short-term experiments, fish were anesthetized and given a single injection of either 0.6% NaCl or the selected hormone in saline solution; they were then permitted to revive for two hours in individual aerated 2000 ml Erlenmeyer flasks, after which each fish was again anesthetized; and blood was taken in the manner described below. The long-term experimentals, which had been receiving saline or hormonal injec-

tions thrice weekly for one month, were similarly anesthetized for blood sampling and autopsied after the last injection. The anesthetic employed was tricaine methane sulfonate (MS 222 Sandoz), 0.04% (1:2500).

Hormonal or saline solutions were injected into the body cavity through the soft skin beside the anus, using a one ml Tuberculin syringe fitted with a 26 gauge needle. The powdered hormones, dissolved in 0.6% NaCl, were of such strength that the desired dose per gram weight of fish was contained in 0.01 ml. The controls received saline injections of 0.01 ml of 0.6% NaCl per gram weight. Thus the volume injected, in both controls and experimentals, was constant in respect to body weight of fish.

The preparations employed, the experiments in which they were used, the activity, the stock solution and the dosage are given in Table I. Stock solutions were prepared in advance and were kept frozen in daily quota until used. A relatively high dose of ACTH (0.75 I.U. gwt) was employed in the acute experiments; a 3/5 smaller dose (0.45 I.U.) was used in the chronic experiment. Chronic treatment with a dose of this order of magnitude has been shown to stimulate the anterior inter-renal tissue of the killifish (Pickford and Atz, 1957). Sections of the head kidney showed that in these fish the anterior inter-renal was strongly stimulated (Kosto, *et al.*, 1959). The cortisol dosage was selected on the following basis: Phillips (1959) has shown that the blood of male killifish contains 15.4 $\mu$g/100 ml serum and that of the females 8.2 $\mu$g. Allowing for a hematocrit of the order of 25% and a total blood volume of 2%, it has been estimated that a dosage of 5 $\mu$g/gwt of fish would result in a circulating level that might be 1000–2000 times greater than normal. Brief warming of cortisol to 45°C was necessary to bring the coarse crystals into solution; the fine precipitate which formed on cooling was then injected as a homogeneous suspension. Repeated injections of methyl-testosterone in the amount of 2 $\mu$g/gwt of fish is known to cause development of nuptial colors and to stimulate the testes of hypophysectomized *F. heteroclitus* (Pickford and Atz, 1957), and a dose of thyrotropin in the amount of 0.4 mU/gwt of fish stimulated the thyroids (Pickford, 1954b).

Hypothyroid fish (Harris, 1959) were obtained by repeated injections of radioactive iodine as follows: 25, 15 and 10 $\mu$C/fish on October 24th and November 27th 1956 and January 4th 1957 respectively. The fish were then kept in salt water until July 4th 1957, when half of each group was transferred to running fresh water. Screening tests to aid in selecting markedly hypothyroid fish were made with tracer doses of $I^{131}$. The thyroids were sectioned to determine the degree of thyroid destruction.

Hypophysectomy was performed in the manner described by Pickford (1953a); the fish used in the experiments reported here were believed to be completely hypophysectomized, as determined by growth failure, by testes regression, and by dissection of the head after autopsy.

In the shock experiments (6, 10) the fish were placed for three minutes

in a beaker of salt water containing ice (0–1°C) and then returned to sea water at laboratory temperature. Complete blood counts were made at intervals of one or two hours after removal of the fish from the ice water.

To ascertain the possible effects of shock in catching and transporting fish from the field to laboratory aquaria (Exp. 18), blood samples were taken in the field at the time of capture, and at the laboratory at 4 and 24 hour intervals after capture. Only males were used.

For the field samples, ten males were captured individually with a hand net and all manipulations on each fish were completed before the next one was caught. Each specimen was dropped immediately into the anesthetic fluid at the scene of capture and blood for hematological study was taken in the usual manner. Two samples were thus diluted with the appropriate solutions for red and white cell counts respectively. The blood in each pipette was analyzed later in the laboratory.

Following completion of the field sampling, 25 males were caught and transported in buckets of brackish water to the laboratory, where they arrived at about 12 noon (transport time *c* 15 min.) and were held in these containers under vigorous aeration. At about 4 PM the same day, four hours after capture, ten of these killifish were killed and sampled. Ten more were killed and sampled the following noon, 24 hours after capture.

In large fish, blood can be readily obtained by cardiac puncture, but in the case of *F. heteroclitus* it was difficult to obtain a sufficient quantity of blood by this method. Several investigators (Katz, 1949; Antipova, 1954) have obtained an adequate flow of blood from the caudal artery by cutting off the tail, and this method was employed with good results in the present investigation. The only difficulty encountered was a greatly reduced flow if the specimen had been anesthetized too long before sampling. At autopsy the animal was lightly anesthetized and carefully dried; the anal fin was then cut off and the tail stem was severed from the body with a heparinized razor blade. The free-flowing blood was dropped on a siliconized slide and then sampled.

To ascertain the validity of the tail-sampling method, a special experiment (Exp. 12, Table III) was performed to compare hematological values of blood taken simultaneously from heart and tail. In some instances the specimens were large enough so that sufficient blood for this experiment could be obtained by standard macro-procedures, but in most cases the specimens were so small that only a micro-procedure could be employed (see below). Since only a limited amount of blood was available, it was considered sufficient to restrict this investigation to red and white cell counts.

Direct cardiac puncture was so difficult as to be impractical in this species and a different procedure was employed to sample heart blood. Scales were scraped from the cardiac region of the throat just behind the operculum so that, by means of a small incision, a triangle of skin and muscular tissue over the pericardium could be removed to expose the heart. Little or no bleeding

ensued. Fluid in the pericardial chamber was absorbed on filter paper and a small speck of heparin powder was sprinkled on the heart. The ventricle was then punctured and the blood was collected in a fine siliconized pipette having a constriction beyond the dilation to help regulate suction. The blood from the heart was then deposited on a siliconized slide and sampled. Immediately thereafter blood was sampled from the tail stem (as described above) while the heart was still beating.

The estimation of hemoglobin has proved more difficult in lower vertebrates than in mammals, mainly because of the nucleated red cells. The acid employed in orthodox methods destroys the cytoplasm of the erythrocytes while leaving the nucleus intact. The cloudy solution which results must be allowed to stand for one hour or more to become clear (Wintrobe, 1933). On the other hand, satisfactory results were obtained with the alkaline method of Sheard and Sanford (1933), as recommended by Schultze and Elvehjem (1934), provided readings were made within 15 minutes; lower readings were obtained with longer standing. Blood taken into a Bureau of Standards hemoglobin pipette to the lower mark was diluted with 0.1% sodium carbonate to the upper mark. The sample was then read in a Klett photoelectric colorimeter with a green filter (wavelength 540 Å) and the result was reported as grams of hemoglobin per 100 ml of blood.

The total red cell count was determined either by the standard macro-procedure or, in special circumstances, by a micro-procedure.

In the macro-procedure, a Bureau of Standards Trenner automatic red blood cell pipette was used. The blood, brought to the first mark, was diluted to the second mark, and the pipette was placed for three minutes in an automatic shaker. The diluting fluid, either Hayem's solution or 3% sodium citrate, was colored with brilliant cresyl blue to facilitate the thrombocyte count. Both sides of the Levy counting chamber, with improved Neubauer double ruling, were filled with this suspension and the cells were allowed to settle for at least 10 minutes. The cells in five small squares on each side of the chamber were counted, and if the results differed by more than 10 cells, the chamber was refilled for another count.

In the micro-procedure, a Lambda micro-dilution pipette[2] was used. This permitted one $mm^3$ of blood to be diluted to 100 $mm^3$. The validity of this method was confirmed by direct comparison with the macro-procedure (Exp. 13, Table IV).

As noted above, the acid used in orthodox methods destroys the erythrocytes in lower vertebrates, and this interferes also with the determination of the white cells. With the cytoplasm destroyed, the nuclei of the erythrocytes resemble lymphocytes, especially when viewed in a counting chamber. The late Dr. L. E. Wolf (personal communication) found that the dilution fluid of

[2] Research Specialities Co., 200 S. Garrard Blvd., Richmond, California.

Rees and Ecker (Todd and Sanford, 1931: 267) could be used on fish blood with satisfactory results, and this method was used in the present investigation.

Total white cell counts were made either by means of a Bureau of Standards Trenner automatic or a regular white blood cell pipette. The procedure was similar to that employed for counting red cells except that the four large corners of the counting chamber were used. Precaution must be taken to wait for at least 10 minutes before the cells are counted, since that amount of time is necessary for the nuclei of the white cells to become properly stained. After 30 minutes the nuclei of the red cells and thrombocytes begin to show a faint coloration, but the deeply stained nuclei of the white cells are still clearly distinguishable.

Thrombocytes were estimated simultaneously with the red cells since, as stated above, the diluting fluid was tinted with brilliant cresyl blue.

Differential counts (lymphocytes and eosinophils) were determined on dried stained smears by the rapid Giemsa method (Slicher, 1951). While still wet, the slide was flooded with 95% ethanol to remove any precipitated stain. The slide was then washed immediately under running tap water and air-dried. Examination under oil immersion followed, with at least 300 cells being counted. Sometimes it was necessary to count cells on two slides in order to satisfy this criterion.

The basic data pertaining to all experiments reported here are contained in Table II.

## ABBREVIATIONS AND FORMULAE

H or Hypect = Hypophysectomized
St = Statistic
GSI = Gonadosomatic Index
Hgb = Hemoglobin in grams per 100 ml blood
RBC = Red blood cell count in millions per cmm
WBC = White blood cell count in thousands per cmm
Eos = Eosinophils in percentage of W.B.C.
Thromb = Thrombocytes in thousands per cmm
N = Normal
B = Breeding
R = Regressed
M = Maturing

A result is significant if the critical ratio (C.R.) is more than 2 when determined by the formula:

$$\text{C.R.} = \frac{\text{mean}_{\text{exp.}} - \text{mean}_{\text{control}}}{\sqrt{(\text{SE}_{\text{exp.}})^2 + (\text{SE}_{\text{control}})^2}}.$$

The GSI, which provides a simple means of evaluating the sexual state, has been calculated from the following formula:

$$\frac{\text{weight of gonads}}{\text{weight of fish}} \times 100.$$

## OBSERVATIONS

### Components of Peripheral Blood

*Red Cells.* Diameters: wet cells, males, $7.5 \times 5.25\,\mu$; females, $8 \times 6.25\,\mu$; wet nuclei, males, $c\ 3.75 \times 2.75\,\mu$; females, $c\ 4 \times 2.75\,\mu$; dry cells, males, $6.75 \times 4.50\,\mu$; females, $7 \times 5\,\mu$; dry nuclei, males, $3.25 \times 2.50\,\mu$; females, $4.50 \times 3\,\mu$.

The cytoplasm of the mature erythrocyte, containing a full complement of hemoglobin, is strongly acidophilic, orange-red in stained smears. The nucleus, often having a spoke-wheel arrangement, is oval in form and frequently has irregular protuberances. Sometimes one or two small red-staining bodies are observed near one or both poles of the nucleus. Non-nucleated red cells, erythroplastids (Nigrelli, 1929), were occasionally observed.

The normoblasts can be divided into three groups: *a.* orthochromatophil; *b.* polychromatophil; *c.* basophil. These distinctions are made with the different characteristics of the nucleus and cytoplasm in view. In *a*, the cytoplasm is acidophilic, the cell has acquired its oval form, and the nucleus is compact. In *b*, the cytoplasm displays basophilic blue areas varying from light to dark, and acidophilic hemoglobin is evident; the nucleus has a definite spoke-wheel arrangement. In *c*, the cytoplasm is homogeneous and intensely blue-staining; the nucleus has chromatin in large clumps and blotches which are interconnected by thick threads. This cell is seen occasionally in the peripheral blood stream, appearing mostly in cases of severe anemia following hypophysectomy.

The hemocytoblast, the youngest cell, is round and has an intensely basophilic blue-staining cytoplasm. The chromatin in the nucleus is fine and delicate and a large nonstaining nucleolus is usually present. This cell, like the basophilic normoblast, is seldom seen in the peripheral blood stream, and it also appears after hypophysectomy.

*White Cells.* Only two types of white cells, both mononuclear, have been observed in the peripheral blood stream of *F. heteroclitus*.

*a.* The first type, here referred to as the lymphocyte, is round ($c\ 6\,\mu$ in diameter) and has a basophilic cytoplasm. The nucleus is compact and fills the greater part of the cell. Small azure-staining granules are sometimes noted in the cytoplasm. Very occasionally larger lymphocyte-like cells are observed.

*b.* The second type, the eosinophil, is granular. This cell, the largest found in the peripheral blood stream, is round or slightly oval (8 to 10 $\mu$ in diameter). The cytoplasm is acidophilic and has specific round red-staining granules. Two types of eosinophils have been noted: the first contains large densely-staining granules which completely fill the cytoplasm between the cell wall and the nucleus; the second type has a much finer granulation, and scattered granules may be seen overlaying the nucleus. The latter type appears in the peripheral blood stream after ACTH and cortisol treatment.

*Thrombocytes.* These cells are spindle-shaped (*c* 7 × 1.5 $\mu$). The cytoplasm is delicate and is present in small quantity, showing mostly at the cell poles only. The nucleus resembles that of the lymphocyte.

## Comparison of Blood Sampling Techniques

### (Table III, Exp. 12; Table IV, Exp. 13)

*Comparison of Heart and Tail Blood Sampling.* Fourteen regressed females were subjected to blood sampling from the heart and tail according to the method described on page 9. As can be seen in Table III, the differences in the values of both red cells (3,760,000 ± 50,000 against 3,790,000 ± 130,000) and white cells (5,420 ± 260 against 5,300 ± 300) were not statistically significant. In fact, the differences were so small that they fell within the limits of experimental error.

*Comparison of Micro- and Macro-techniques of Blood Sampling.* Twelve regressed females served for this comparison (see p. 10). As can be seen in Table IV, no statistical difference was established in the number of either red cells (macro: 3,810,000 ± 150,000; micro: 3,830,000 ± 130,000) or white cells (macro: 6,200 ± 920; micro: 6,320 ± 940). As above, the differences were within the limits of experimental error.

## Effects of Capture and Transport and of Submersion in Ice Water

### (Table V, Exp. 18; Table VI, Exps. 6, 10)

*Effects of Capture and Transport.* Blood samples were taken in the field immediately upon capture and in the laboratory approximately 4 and 24 hours later (Exp. 18). The red cell count was slightly but not significantly lower in fish held for four hours than in those sampled at the time of capture, but in specimens held for 24 hours the number had risen markedly. Unfortunately, in this experiment, limited aquarium facilities precluded the maintenance of parallel groups for longer periods of time, and comparisons must therefore be made with other experiments (see Discussion). The white cell count was extremely low at the time of capture and had dropped still further four hours later (not

significant), but after 24 hours it had increased significantly toward normal laboratory levels; nevertheless, this count was still less than that in aquarium fish held for longer periods, either in summer or winter. The thrombocytes, initially low, showed a striking increase after four hours; no data were obtained for those held for 24 hours.

*Effects of Cold Shock.* Exps. 6 and 10 were performed with a total of 30 regressed normal males. The procedure is given on pp. 8, 9. No effect on the hemoglobin and red cell count could be noted one hour after submersion in ice water. However, a significant decrease in the number of white cells was found: 19.4% in Exp. 6, 34.1% in Exp. 10. A 50% decrease in eosinophils was evident in both experiments. In both cases there was a nonsignificant increase in thrombocytes (19.6% and 5.4%).

After two hours the picture was different. The hemoglobin and red cell values had dropped significantly: hemoglobin 18.1 and 8.7%, red cells 25.8 and 12.1% respectively in Exps. 6 and 10. In both experiments the thrombocytes increased, but only in Exp. 6 was the increase statistically significant. A highly significant increase in the number of white cells – 84.6% and 89.7% – was apparent in both Exps. 6 and 10 respectively, with a concomitant rise in the number of eosinophils (100% and 75%).

It was noted that the blood two hours after shock contained a large number of disintegrated red cells.

## Peripheral Blood in Normal Recently-captured and Aquarium-kept Fish

(Table VII, Exps. 1, 5; Table VIII, Exps. 3, 8, 15)

*Recently-Captured Specimens.* In Exp. 1, observations on 16 males and 16 females in early summer during the breeding season, approximately two to six hours after capture, showed that there is a definite difference in hematological values between the sexes (Fig. 1). The hemoglobin of females was 22.9% lower than that of males and the red cell count was 32.7% lower; both of these differences were statistically significant. The total white cell count as well as the thrombocytes were slightly and nonsignificantly lower in females than in males: 7.9% and 9.8% respectively. Only the eosinophil values were reversed, with females having the higher number, the difference being 36.4% (not significant).

In Exp. 5, 24 regressed males and females were examined during the winter, approximately two to six hours after capture. In this instance the hemoglobin, white cells and thrombocytes in females were significantly higher than those in males (4.8%, 15.2%, 25.3% respectively) while the red cell counts in the two sexes were identical (Fig. 1). However, in males the eosinophils were slightly but not significantly higher (9.1%) than in females.

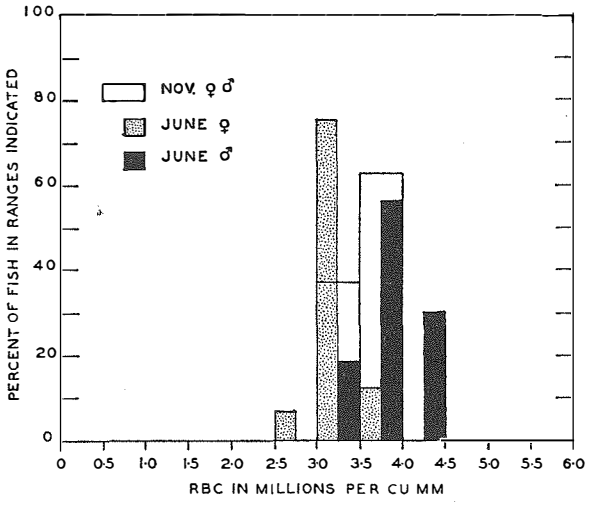


Figure 1. Sexual differences in erythrocyte counts during the breeding season compared with the common intermediate value during regression in male and female *F. heteroclitus* (Exps. 1, 5).

When seasonal differences in males are studied, it is seen that all values decreased from summer towards winter: hemoglobin 15.1%; red cells 22.6%; eosinophils 44%; and thrombocytes 7.5%; the white cells remained essentially the same. On the contrary, females showed a reverse trend with increases from early summer to winter, except for the eosinophils: hemoglobin 9.0%; red cells 8.3%; white cells 25.4%; and thrombocytes 36%; only the eosinophils showed a decrease, 63.3%. Except for the white cells and thrombocytes in males, all of these differences were statistically significant.

In stained blood smears from both experiments, most of the white cells were lymphocytes, the remainder eosinophils. The red cells appeared mature and oval in shape with an elongated nucleus, but the long axis of the nucleus did not always coincide with that of the cell. During the breeding season (Exp. 1) many normoblasts were present.

*Aquarium-kept Fish.* In Exp. 8, 24 regressed males and females were kept for 3½ months in salt water during winter, and no statistical differences in hematological values were observed between the sexes. Only a slight difference was noted in the white cell and eosinophil values, with females showing the higher numbers. The blood picture of a parallel group (11 ♂; 13 ♀) in fresh water instead of salt water showed a similar absence of differences; in this case the eosinophil count was significantly higher in the females.

In comparing the fresh and salt water groups, only minor differences were noted; in both sexes of the fresh water group, the hemoglobin and red cells showed a slight trend toward higher values while the white cell count was

significantly higher and the eosinophil percentages significantly lower. Although thrombocyte numbers were similar, they still were slightly higher for fish in fresh water.

The stained blood smears of fish kept in fresh water showed the same two types of white cells noted before, namely eosinophils, small lymphocytes, and an occasional large lymphocyte. The red cells were irregular in size and shape and in the position of the nucleus. Many erythrocytes had indented nuclei, a large number of which were off-center and touched the periphery of the cell.

In Exp. 15, 53 males and females, captured in autumn, were kept in either salt or fresh water until the approach of the breeding season the following spring. For six months they were illuminated for eight hours daily, but during the last month the illumination was increased to 17 hours.

Comparison of the values from the fresh water group with those from the salt water group provides the following general picture. In the salt water specimens, the hemoglobin and red cell values were statistically higher in males than in females, but the eosinophils, though higher also, were not statistically significant; on the other hand, the white cells and thrombocytes were significantly higher in the females. In the fresh water group the same general picture was obtained, but here the differences were less pronounced than in those from salt water.

Comparison of specimens from fresh water with those kept in salt water shows the following results. Among the males, those in fresh water had lower values in hemoglobin (14%, significant), red cells (15.4%, significant), and eosinophils (21.4%, significant) but significantly higher values in white cells and thrombocytes (29.3% and 15.3% respectively). In a similar comparison of data for females, the general trend was the same as that for males, excepting thrombocytes: hemoglobin in fresh water females was 3% less (not significant) than that in salt water specimens, red cells 7% less (significant), eosinophils 25% less, white cells 25% more, and thrombocytes 5.1% less. Note that the data for hemoglobin and red cell counts in Exp. 15 show a reversal of the trend observed in Exp. 8 and that only hemoglobin shows a reversal in Exp. 3 (below). The morphology of the blood elements was essentially the same as that noted in Exp. 8.

In Exp. 3, five breeding males were kept in only salt water from August 1956 to July 1957 while seven similarly treated specimens were transferred from salt to fresh water for the last 16 days of the experiment. Here the hemoglobin, white cells, and thrombocytes of specimens transferred to fresh water were higher than those from the salt water controls while the red cell and eosinophil values were slightly lower. No differences were significant. These values may also be compared with those of Exps. 8 and 15. Hypothyroidism (p. 25) heightened the effect on the white cells.

The red cells were irregular in shape and size, many having indented nuclei. Non-nucleated red cells (erythroplastids) were present in small numbers.

### Effects of hypophysectomy on Blood Components

(Table IX, Exps. 2, 8, 17, 19)

Data from these experiments provide means of comparing uninjected males and females which had been hypophysectomized for $5^1/_2$ to 10 weeks with normal regressed and breeding specimens, all groups having been kept in salt water.

When hypophysectomized males (Exp. 2) were compared with regressed specimens (Exp. 8), lower values were observed in hemoglobin (21.5%), red cells (20.1%) and white cells (49.1%); the thrombocytes remained within experimental limits and eosinophils were lacking.

Stained smears revealed that the agranular leucocytes were similar to those observed in normal blood; these lymphocytes were mostly small, but an occasional large one was seen. The most notable change was in the erythrocytes, where uniformity was no longer present; their sizes differed and their shapes ranged from extreme oval to round (old to young cells). Several erythroplastids were seen. Different types of nuclei were observed and in a few cases, where extreme anemia was present, cells in active amitotic division were found in the peripheral blood. The color of the cytoplasm ranged from yellowish-pink to lead-blue.

The same 12 regressed males used above (Exp. 8) may be compared with another hypophysectomized group (Exp. 17); the same experiments provided comparable data on females. All groups had been kept in salt water. In April or May, when the gonads began to mature, the pituitaries were removed and autopsies were performed in June. Lower significant values were found in the hypophysectomized males, excepting thrombocytes, which were higher but not significantly so. The same picture was obtained for the females, but in this instance the thrombocytes also were significantly lower in the hypophysectomized females. After hypophysectomy, a few eosinophils were observed in the circulating blood from 10 of the 20 fish studied, irrespective of sex.

Also compared are data for: 10 normal breeding males (autopsied in July) from Exp. 19 with the 10 hypophysectomized males from Exp. 17 used above; and 10 normal breeding females from Exp. 19 with the 10 hypophysectomized females from Exp. 17 used above. For males the results showed the same pattern as that noted in comparing data from Exps. 8 and 17, although many of the absolute values were different. In females the hemoglobin and red cell values, which are already low during the breeding season, were not greatly changed through hypophysectomy ($5^1/_2$–7 weeks), nor was there much difference in thrombocytes; however, as in the previous comparison for males, the white cell and eosinophil counts were low in the hypophysectomized females.

## Effects of Saline and Hormone Injections in Normal and Hypophysectomized Fish

*Saline Solution* (Table X, Acute Exps. 4, 7, 10, 11, 14, 17, 19; Chronic Exps. 2, 9). The results of single injections of 0.6% NaCl are summarized in Table XVI. In the first group, four injected normal regressed males were compared with six uninjected controls. Two hours after injection there was a positive but not significant increase in the hemoglobin and red cell count; however, there was a significant decrease in the white cells (21.9%) in the injected specimens, a doubling in the number of eosinophils, and a slight increase in thrombocytes. An experiment with normal regressed females (Exp. 14) gave essentially similar results: the hemoglobin showed a statistically significant increase (27.3%), there was again a marked decrease in the white cell count (31.6%) but, unlike the males, there was no increase in eosinophils.

Exp. 19 was performed on similarly injected and uninjected males and females, but these were in the breeding state. No significant changes were observed in injected fish of either sex except in respect to the eosinophils, which increased markedly (♂: 137%; ♀: 377%). The males provided more or less similar results to those of Exps. 10 and 11 except that in Exp. 19 the white cells did not drop significantly, as they did in the previous experiments. The summer females were less reactive than the winter females (Exp. 14); neither the increase in hemoglobin nor the decrease in leucocytes was statistically significant; however, they showed much higher eosinophil values.

No changes were observed in the morphology of the blood elements.

In Exps. 2, 4, 7, and 17, parallel studies to those above were carried out with hypophysectomized fish instead of normal specimens. As above, these were injected with 0.6% NaCl and were autopsied two hours thereafter, except in Exp. 2 which provided uninjected controls for Exps. 4 and 7. However, the periods of hypophysectomy varied as follows: Exp. 2 – ten weeks; Exp. 4 – six months; Exp. 7 – two months; and Exp. 17 – 5½ to 7 weeks.

In Exps. 4 and 7 combined, the injected males on an average provided higher values for all blood components, excepting thrombocytes, which were significantly lower (26.8%). Neither red cells nor hemoglobin showed statistically significant changes, but the increase in the white cells was significant (70.7%). Eosinophils reappeared in the blood stream.

The injected males of Exp. 17 showed a similar pattern, but here the hemoglobin and red cells gave significant increases of 16.5 and 13.9% respectively; the white cells increased insignificantly (17.7%) while the eosinophils, still present in some of the controls, decreased or disappeared; the thrombocytes, as in Exps. 4 and 7, decreased significantly (14.4%). In the females of Exp. 17, the picture was different from that of the males. The hemoglobin

and red cells decreased insignificantly while the white cells increased insignificantly (34.4%); the eosinophils doubled in the injected specimens and the thrombocytes increased significantly (17.3%).

The study of stained smears showed no differences in the morphology of cell types which were typical of hypophysectomized controls (see p. 17).

*ACTH* (Table XI, Exps. 2, 4, 11, 17, 19). The results of experiments with ACTH (acute experiments) are summarized in Table XVII.

Two types of experiments were performed to evaluate the effects of ACTH on the circulating blood elements: A. Killifish were injected with a single high dose of ACTH and autopsied two hours after injection; results are summarized in Table XVII. B. Fish were injected thrice weekly for one month with various ACTH preparations (see Table I) and autopsied 24 hours after the last injection.

In Exps. 4 and 11 on normal regressed males, 10 specimens received 0.6% NaCl injections, the other 10 ACTH (Prep. 1b in Exp. 4, Prep. 1c in Exp. 11). Two hours after a single high dose of ACTH, a significant decrease was noted in both hemoglobin (9.7% in Exp. 4, 9.3% in Exp. 11) and red cells (9.2% in Exp. 4, 11.6% in Exp. 11). The number of white cells was markedly elevated (five-fold in Exp. 4, six-fold in Exp. 11), and the number of eosinophils was more than doubled in both experiments. The thrombocyte results were contradictory, there being a significant increase in Exp. 4 and a significant decrease in Exp. 11.

The stained blood smears also presented a different picture after ACTH treatment as compared with the saline-injected controls. Two different types of eosinophils were noted, one with heavy granulation, the other with finer granules. In all cases the nucleus was acentric. The red cells were in different stages of maturation, and many nuclei from disintegrating red cells were present. Hemocytoblasts were present in moderately high numbers (7%).

In Exp. 19, normal breeding males as well as females were treated in a manner similar to that in Exps. 4 and 11. Prep. 1c was used in this experiment. Results from breeding males confirmed those obtained from regressed males, excepting eosinophils, which in this case decreased instead of increasing with ACTH treatment. Females responded somewhat differently from the males, with statistically insignificant increases in hemoglobin and red cell counts; the striking 7.5-fold rise in white cells was not very dissimilar to the pronounced increase noted in the males of Exp. 11. Eosinophils changed little while the thrombocytes increased noticeably.

The different cell types were similar to the ones described above.

Exp. 4 included a group of male fish which had been hypophysectomized for a period of six months and which received 0.6% NaCl or a large dose of ACTH (Prep. 1b) two hours before autopsy. Here a significant decrease was noted in hemoglobin (23.7%), red cells (20.5%), and eosinophils (70%); the

number of white cells more than doubled (2.5 times), and the thrombocytes increased 22.5%.

In Exp. 17, both male and female hypophysectomized fish received the same treatment as those in Exp. 4, except that Prep. 1c was used and the fish were hypophysectomized for only 5½–7 weeks. The response of the ACTH males was different from those in Exp. 4: that is, a slight increase was obtained in hemoglobin (3%) and only a slight decrease in red cells (1.8%); the white cell increase was still high while the thrombocytes changes little. In the females, hemoglobin rose a significant 12.9%, the red cells 17.8%. The most striking results in this experiment were the pronounced increases in white cells (10 times in males, 20 times in females) and in the number of eosinophils in both sexes (*cf* Exp. 4).

The stained blood smears also revealed considerable change. A great difference was noted, such as that described for normal fish in Exps. 4 and 11, but in this case the effect of ACTH on red cells was more marked than in normal animals receiving this hormone; the cells were in different stages of maturation, with an average of 20% young erythrocytes (normoblasts). A great number of disintegrating cells were also present.

The changes in normal and hypophysectomized males were fairly similar in respect to hemoglobin, red cell and white cell counts but varied considerably in respect to eosinophils and thrombocytes; the normal and hypophysectomized females likewise produced similar results, excepting thrombocytes, which were decreased in the hypophysectomized fish.

Exp. 2 compares data from nine uninjected hypophysectomized males with ten similar males that had received $\alpha_s$-ACTH (Prep. 1a) thrice weekly for one month, with autopsies 24 hours after the last injection. Compared with the uninjected controls, the experimentals showed a significant increase in all constituents, excepting thrombocytes, which remained at about the same level.

Hemoglobin increased 39.3%, the red cells 41.7%; the total number of white cells went up 2.5 times and the eosinophils rose from zero to 4.4%. Effects of hypophysectomy and chronic treatment with ACTH are shown in Fig. 2.

The morphology of the cellular elements in the stained slides was as described above.

The hemoglobin and red cell increases here were much higher than they were in the other ACTH males, and the white cell increases, as in most of the other males, were highly significant.

*Cortisol* (Table XII, Exps. 7, 14, 16, 17, 19). The results of experiments with cortisol are summarized in Table XVIII. In the normal regressed males of Exp. 7, comparison with saline-injected specimens shows that a single high dose of cortisol two hours before autopsy resulted in an insignificant rise of

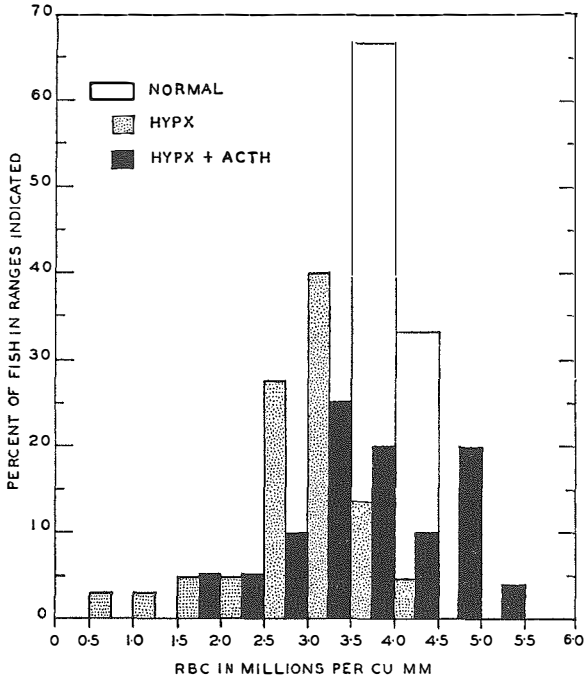


Figure 2. The effects on the erythrocyte count of hypophysectomy and replacement therapy with chronic injections of ACTH in comparison with normal regressed *F. heteroclitus*. Normal controls: males and females kept in the laboratory in salt water (Exp. 8). Hypophysectomized controls: uninjected (Exp. 2), acute saline injected (Exp. 4 and 7), and fish receiving chronic injections of saline, prolactin, intermedin or a combination of prolactin and intermedin (Exps. 2, 9). None of these treatments had any significant effect on the red cell count. ACTH: fish receiving chronic injections of ACTH or ACTH plus prolactin (Exp. 2).

hemoglobin (10.2%), a significant increase in red cells (21.2%), significant drops in white cells (33.3%) and eosinophils (68%), and little change in thrombocytes, the last remaining within the range of experimental error.

Exp. 19, similar to the foregoing, was performed on normal breeding males. These specimens showed significant decreases in hemoglobin (9%) and red cells (11.7%), a rise in white cells (2.5 times), almost a doubling in the number of eosinophils, and a slight drop in the thrombocytes. Thus, compared to the data above from Exp. 7, there was a reversal in the direction of change in all blood components.

The normal regressed females of Exp. 14, with treatment similar to that of the males above, showed a significant decrease in hemoglobin (12.6%), a slight and insignificant increase in red cells (1.7%), a significant decrease in white cells (37%), a pronounced significant eosinophil decrease (92%), and a significant 26.8% drop in thrombocytes. These results, compared to those

of the regressed males (Exp. 7), showed essentially similar results except for the hemoglobin and thrombocyte decreases.

Stained smears revealed the same type of response which was noted in the ACTH experiments. The red cells were diverse in size and shape (oval to round) and the nuclei were polymorphic, many being bean-shaped. The lymphocytes were of the small variety, and an increase in the percent of hemocytoblasts was apparent. Again two types of eosinophils, with both coarse and fine granulation, were present.

Exp. 16 is especially interesting in that it was performed on normal maturing males and females in May, at which time the condition of the gonads in both sexes is highly variable, ranging between individuals which are still far from maturity to those which exhibit flowing sperm or discharged eggs. In this case, where maturity ranges widely, means for the gonosomatic index are meaningless, hence the values for each individual specimen are listed at the end of Table XII. Here, as in the three preceding experiments, all specimens except two females received injections of either 0.6% NaCl or cortisol two hours before autopsy. The cortisol injections in males produced a rise in hemoglobin whereas in females it induced a decrease. The red cells increased in the males but in the females they decreased. The response of the leucopoietic tissues depends on the state of maturation of the gonads in both sexes (Fig. 3); in two males (385, 386) with a low GSI the white cell counts were lower than the mean for saline controls whereas in four with flowing sperm (381, 382,

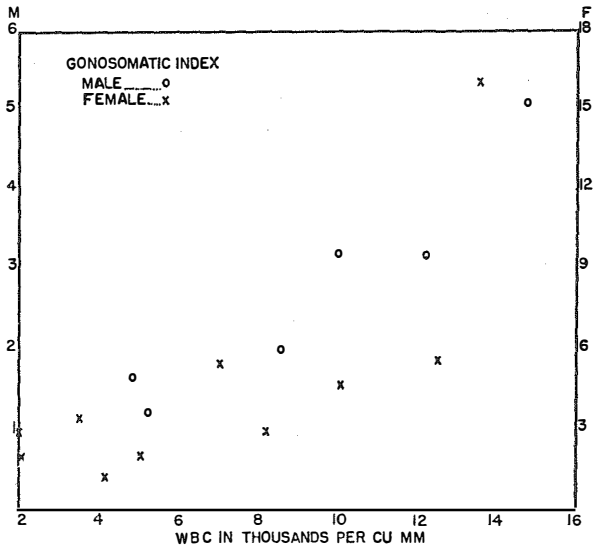


Figure 3. Correlation of the response of the white blood cell count to a single injection of cortisol in relation to the state of sexual maturation in male and female *F. heteroclitus* (Exp. 16).

383, 384) the white counts were markedly increased. Similarly, in five females with immature ovaries (365, 368, 370, 371, 372) the white cell counts were lower than the mean for saline controls whereas more mature females showed higher values. The marked increase in eosinophils resulting from saline injections was not further increased by cortisol treatment in the females, but in males it was greater. Thrombocytes remained on the same level.

In a pilot experiment, No. 7, a single large dose of cortisol in hypophysectomized male fish two hours before autopsy produced a significant decrease in hemoglobin (8.8%), no change in the red cell count, a statistically significant three-fold increase in white cells, a five-fold rise in eosinophils, and a significant increase in the thrombocytes (30.7%). Here we note a decrease in hemoglobin and little change in red cells as compared to the increase in these components in the normal regressed males. Also, we see pronounced increases in white cells, eosinophils and thrombocytes compared to significant decreases in the former two and only a slight increase in thrombocytes.

In Exp. 17, hypophysectomized fish of both sexes were used, the duration of hypophysectomy being $5^1/_2$–7 weeks. The response of these males to cortisol provided a pattern similar to that of hypophysectomized males in Exp. 7, discussed in the preceding paragraph. However, in these fish the increase in white cells was much greater (4.5 times in Exp. 17; only 3 times in Exp. 7); the red cell count was significantly decreased, which was not the case in Exp. 7; and eosinophils and thrombocytes were much increased, both being highly significant. The effect of this steroid on similarly treated hypophysectomized females was slightly different. The hemoglobin increased only slightly (3.3%) and the red cell count remained the same; there was a marked increase in the number of white cells (10 times) and eosinophils (15 times), and the thrombocytes increased a significant 37.5%.

As is seen above, the response of hematopoietic organs differs in the hypophysectomized sexes: when hemoglobin and red cell values are compared, the effect is opposite, especially in the case of hemoglobin. For the other constituents, the effect is similar; but clearly, under the influence of cortisol, the females responded much more significantly than did the males.

*Prolactin, ACTH, Prolactin and ACTH, Intermedin, and Prolactin and Intermedin* (Table XIII, Exps. 2, 9). Exps. 2 and 9 were performed on hypophysectomized males, and each subject, excepting the nine controls in Exp. 2, was injected thrice weekly for one month with 0.6% NaCl, prolactin, $\alpha_s$-ACTH (Prep. 1 a), prolactin + $\alpha_s$-ACTH, intermedin, or prolactin + intermedin.

With prolactin alone, the increases in hemoglobin values were insignificant (4.5%) in Exp. 2 but significant (11.4%) in Exp. 9; likewise, prolactin plus intermedin in Exp. 9 had a positive effect with an 18.3% increase; in these same instances the red cells showed insignificant increases (3.7% in Exp. 2;

4.5% and 16.8% in Exp. 9). White cells in Exp. 2, with prolactin alone, decreased insignificantly (19.6%) whereas in Exp. 9, with prolactin alone and with prolactin and intermedin, there was a significant increase in both instances. With prolactin alone, eosinophils appeared in the blood stream in both experiments, but with prolactin plus intermedin there was no change. The thrombocytes varied without a definite trend.

Intermedin alone (Exp. 9)[3] induced a significant decrease in hemoglobin (15.3%), an insignificant decrease in red cells (7.4%), a significant increase in white cells (30.5%), no change in eosinophils, and a significant increase in thrombocytes (17%).

Results from $\alpha_s$-ACTH alone (Exp. 2) provided a significant increase in all blood components except thrombocytes: hemoglobin 39.2%, red cells 29.4%, and white cells 56.6%. ACTH in combination with prolactin provided parallel results, but in this case the red cell and eosinophil increases were greater (hemoglobin 24.4%, red cell 13%, and white cells 37.3%) while hemoglobin and white cells were somewhat less; the thrombocytes did not vary greatly.

When studying the stained smears, no striking differences were observed in either white or red cells except in the case of ACTH, discussed above.

*Thyrotropin and Methyl-Testosterone* (Table XIV, Exp. 20). Males, hypophysectomized 6½–8 weeks prior to autopsy, were injected with 0.6% NaCl, TSH, and methyl-testosterone thrice weekly for one month. In the specimens treated with TSH, all hematological values showed a marked increase, with only the white cell and eosinophil increases being insignificant: hemoglobin 15.2%, red cells 27.1%, white cells 13.8% and thrombocytes 25.2%. In the controls, only one fish out of five had one eosinophil in 300 white cells. In fish receiving TSH, two out of eight had an occasional eosinophil.

Within the above-noted period of treatment, methyl-testosterone caused a marked increase in the relative size of the testes, and all the specimens displayed full breeding colors. All hematological values increased significantly excepting the white cells and eosinophils: hemoglobin 13.5%, red cells 51.0%, white cells 14.5%, and thrombocytes 33.7%. Eosinophils were found in only two fish.

It seems likely that neither TSH nor methyl-testosterone trigger a release of eosinophils into the blood stream.

## Hypothyroidism (Table XV, Exp. 3)

Consider now the effect of hypothyroidism in breeding males kept in either salt or fresh water for more than two weeks. In specimens that had been kept in sea water only, there was an insignificant decrease in all blood components

3 There was evidence (Kosto, *et al.*, 1959) that this preparation partially deteriorated during storage of the frozen solution. However, the combination of prolactin + intermedin was active in respect to the stimulation of new pigment cell proliferation.

excepting white cells, which decreased significantly: hemoglobin 20%, red cells 10.9%, white cells 37.1%, and eosinophils 2.5%. In specimens from fresh water the same general picture obtained, but in this case the hemoglobin and thrombocytes instead of the white cells showed significant decreases: hemoglobin 14.3%, red cells 11.6%, white cells essentially unchanged, eosinophils 2.5%, and thrombocytes 13.3%. The only other observation of interest was the enhancement of leucocytosis in the hypothyroid fish kept in fresh water.

In the stained material there was a noticeable difference between normal blood and the blood of hypothyroid fish. In the latter (both salt and fresh water), a large amount of debris (disintegrating erythrocytes) was found. The red cells were irregular in shape, and several erythroplastids were in evidence. Many young red cells were present and many had a double nucleus, but there was no evidence of actual division.

TABLE I. HORMONE PREPARATIONS.

| Name | Experiment | Activity | Stock Solution in 0.6% NaCl | Dose/gwt |
|---|---|---|---|---|
| PITUITARY HORMONES | | | | |
| 1. Adrenocorticotropin | 2 | *c* 150 I.U./mg | 0.03% | .45 I.U. |
| a. $\alpha_8$-ACTH, sheep (C.H. Li*) | | | | |
| b. ACTH, hog (Armour, Acthar Lot K52204) | 4 | – | .67 ml added to 50 I.U. | .75 I.U.<br>.75 I.U. |
| c. ACTH, hog (Armour, Lot 212–103) | 11, 17, 19 | *c* 2.0 I.U./mg | 38 mg/ml | .76 I.U. |
| 2. Prolactin, sheep (C. H. Li†) | 2, 9 | 35 I.U./mg | 0.1% | .35 I.U. |
| 3. Intermedin, pig $\beta$-MSH (I. I. Geschwind, Lot CCD-4T) | 9 | *c* $4 \times 10^9$ Lerner MSH units/g | .15% | *c* 6000 MSH units |
| 4. Thyrotropin, beef, TSH (Armour, Lot 2R3) | 20 | 0.4 U.S. units/mg | 1 mg/10 ml | 0.4 mU |
| STEROID HORMONES | | | | |
| 5. Cortisol (Merck [Sharp & Dohme]) | 7, 14, 16 17, 19 | – | 1 mg/2 ml (brief warming, etc.) | 5 $\mu$g |
| 6. Methyl-testosterone (Schering Corp.) | 20 | – | 2 mg/10 ml | 2 $\mu$g |

* Prepared after the method of Li, *et al.* (1954).
† Prepared after the method of Cole and Li (1955).

TABLE II. BASIC DATA ON ALL EXPERIMENTS.

| Exp. No. | Specimens Normal** ♂ | Normal** ♀ | Hypect. ♂ | Hypect. ♀ | Capture | Dates and Length of Hypect.$\pi$ | Blood Sample | Comments on Treatment†† | Table Reference |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 16B | 16B | – | – | VI/23/57<br>VI/30/57 | –<br>– | VI/23/57<br>VI/30/57 | Freshly caught fish sampled in the laboratory 2–6 hours after capture | VII |
| 2* | – | – | 39 | – | IV/23/57 | V/3–6/57<br>(6 wks) | VII/13–14/57 | Injected thrice weekly for one month, beginning VI/15/57<br>( 9) uninjected<br>(10) Prolactin<br>(10) $\alpha_S$–ACTH (Prep. 1a)<br>(10) Prolactin + $\alpha_S$–ACTH (Prep. 1a) | IX, X, XI, XIII |
| 3† | 28B | – | – | – | VIII/56 | – | VII/20–21/57 | (12) Controls – untreated<br>(5) salt water<br>(7) fresh water after VII/4/57<br>(16) Hypothyroid – treated with $I^{131}$<br>(7) salt water<br>(9) fresh water after VII/4/57 | VIII, XV |
| 4 | 10R | – | 8 | – | IV/23/57 | IV/29–V/6/57<br>(6 mo) | X/31/57 | Autopsies 2 hours after single injection<br>(6) Normal – 0.6% NaCl<br>(4) Normal – ACTH (Prep. 1b)<br>(4) Hypect. – 0.6% NaCl<br>(4) Hypect. – ACTH (Prep. 1b) | X, XI |
| 5 | 12R | 12R | – | – | XI/4/57 | – | XI/4/57 | Freshly caught fish sampled in the laboratory 2–6 hours after capture | VII |
| 6 | 12R | – | – | – | VIII/30–IX/5/57 | – | XI/11/57 | Placed in ice water for 3 minutes and sampled 1 and 2 hours later.<br>Controls untreated | VI<br>(cont.) |

TABLE II. (cont.)

| Exp. No. | Specimens Normal** ♂ | Normal** ♀ | Hypect ♂ | Hypect ♀ | Capture | Dates and Length of Hypect.π | Blood Sample | Comments on Treatment | Table Reference |
|---|---|---|---|---|---|---|---|---|---|
| 7 | 8R | – | 10 | – | VIII/30–IX/25/57 | IX/25/57 (2 mo) | XI/21/57 | Autopsied 2 hours after single injection<br>(4) Normal – 0.6% NaCl<br>(4) Normal – Cortisol<br>(5) Hypect. – 0.6% NaCl<br>(5) Hypect. – Cortisol | X, XII |
| 8 | 23R | 25R | – | – | VIII/30–IX/5/57 | – | XII/3–4/57 | Kept 3½ months in either salt or fresh water from time of capture | VIII, IX |
| 9* | – | – | 40 | – | VIII/30–IX/5/57 | IX/25/57 (2 mo) | XII/19–20/57 | Injected thrice weekly for one month, beginning XI/20/57<br>(10) 0.6% NaCl<br>(10) Prolactin<br>(10) Intermedin<br>(10) Prolactin + Intermedin | X, XIII |
| 10 | 18R | – | – | – | VIII/30–IX/5/57 | – | I/16–17/58 | Placed in ice water for 3 minutes and sampled 1 and 2 hours later.<br>Controls untreated | VI, X |
| 11 | 10R | – | – | – | VIII/30–IX/5/57 | – | I/17/58 | Autopsied two hours after single injection<br>(4) 0.6% NaCl<br>(6) ACTH (Prep. 1c) | X, XI |
| 12 | – | 14R | – | – | IX/15/57 | – | I/23/58 | Heart blood compared with tail blood | III |
| 13 | – | 12R | – | – | IV/23/57 | – | I/30/58 | Macro- and micro-methods of sampling compared | IV |
| 14 | – | 17R | – | – | IX/4/57 | – | II/12/58 | (6) Uninjected<br>(5) 0.6% NaCl<br>(6) Cortisol | X, XII |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 15 | 24M | 29M | – | – | IX/4/57 | – | IV/24–30/58 | Seven months in salt or fresh water from time of capture; 8 hours daily illumination for 6 months, 17 hours daily for last month | VIII |
| 16 | 11M | 22M | – | – | IV/18–30/58 | – | (♀) V/19/58<br>(♂) V/29/58 | (2 ♀) Untreated<br>(5 ♂; 10 ♀) 0.6% NaCl<br>(6 ♂; 10 ♀) Cortisol | XII |
| 17 | – | – | 40 | 40 | IV/15–30/58 | IV/29–V/9/58 ($5^1/_2$–7 wks) | VI/17–19/58 | Autopsied 2 hours after single injection<br>(10 ♂; 10 ♀) Uninjected<br>(10 ♂; 10 ♀) 0.6% NaCl<br>(10 ♂; 10 ♀) ACTH (Prep. 1c)<br>(10 ♂; 10 ♀) Cortisol | IX, X, XI, XII |
| 18 | 30B | – | – | – | VI/25/58 | – | VI/25–26/58 | Freshly caught fish sampled in the field and 4 and 24 hours later in the laboratory | V |
| 19 | 40B | 30B | – | – | VI/24/58 | – | VII/15–17/58 | Autopsied 2 hours after single injection<br>(10 ♂; 10 ♀) Uninjected<br>(10 ♂; 10 ♀) 0.6% NaCl<br>(10 ♂; 10 ♀) ACTH (Prep. 1c)<br>(10 ♂; – ) Cortisol | IX, X, XI, XII |
| 20 | – | – | 21 | – | IV/15/58 | IV/29–V/9/58 ($6^1/_2$–8 wks) | VII/22–24/58 | Injecting thrice weekly for one month beginning VI/25/58<br>(5) 0.6% NaCl<br>(8) Thyrotropin<br>(8) Methyl-testosterone | XIV |

** B = Breeding; R = Regressed; M = Maturing at time of autopsy.
$\pi$ Date of hypophysectomy and period of time between removal of pituitary and beginning of experiment.
†† Figures in parentheses = number of fish used.
* Melanogenesis experiment (Kosto, *et al.*, 1959).
† Hypothyroid experiment (Harris, 1959).

TABLE III. COMPARISON OF HEART- AND TAIL-BLOOD SAMPLING.

| Exp | Spec | Source | St | GSI | Hgb | RBC | WBC | Eos | Thromb |
|---|---|---|---|---|---|---|---|---|---|
| 12 | 14 ♀ | Heart | M | – | – | 3.76 | 5.42 | – | – |
| | N–R | | $s_e$ | – | – | 0.05 | 0.26 | – | – |
| | | Tail | M | – | – | 3.79 | 5.30 | – | – |
| | | | $s_e$ | – | – | 0.13 | 0.30 | – | – |
| | | | *CR* | – | – | *+0.14* | *—0.20* | – | – |

TABLE IV. COMPARISON OF MICRO- AND MACRO-ESTIMATIONS IN BLOOD SAMPLING.

| Exp | Spec | Method | St | GSI | Hgb | RBC | WBC | Eos | Thromb |
|---|---|---|---|---|---|---|---|---|---|
| 13 | 12 ♀ | Macro | M | – | – | 3.81 | 6.20 | – | – |
| | N–R | | $s_e$ | – | – | 0.15 | 0.92 | – | – |
| | | Micro | M | – | – | 3.83 | 6.32 | – | – |
| | | | $s_e$ | – | – | 0.13 | 0.94 | – | – |
| | | | *CR* | – | – | *+0.11* | *+0.92* | – | – |

TABLE V. EFFECTS OF CAPTURING AND HANDLING SPECIMENS, WITH BLOOD SAMPLES TAKEN AT THE TIME OF CAPTURE AND AT FOUR AND TWENTY-FOUR HOURS AFTER CAPTURE.

| Exp | Spec | Time of sampling | St | GSI | Hgb | RBC | WBC | Eos | Thromb |
|---|---|---|---|---|---|---|---|---|---|
| 18A | 10 ♂ | When | M | – | – | 3.35 | 2.03 | – | 91.0 |
| | N–B | captured | $s_e$ | – | – | 0.09 | 0.21 | – | 8.2 |
| 18B | 10 ♂ | 4 hours | M | – | – | 3.22 | 1.84 | – | 258.0 |
| | N–B | after capture | $s_e$ | – | – | 0.14 | 0.19 | – | 10.5 |
| 18C | 10 ♂ | 24 hours | M | – | – | 3.87 | 4.32 | – | – |
| | N–B | after capture | $s_e$ | – | – | 0.10 | 0.17 | – | – |
| | *Cf* B with A | | *CR* | – | – | *—0.8* | *—0.7* | – | *+12.5* |
| | *Cf* C with A | | *CR* | – | – | *+3.8* | *+9.5* | – | – |

TABLE VI. Effects of Cold Shock Determined by Placing Normal Regressed Males in Ice Water for Three Minutes and Sampling the Blood One and Two Hours after Removal from Ice Water.

| Exp | Spec | Autopsy | St | GSI | Hgb | RBC | WBC | Eos | Thromb |
|---|---|---|---|---|---|---|---|---|---|
| 6A | 4 ♂ | Without | M | 0.40 | 10.2 | 4.57 | 6.50 | 7.0 | 117.0 |
| | N–R | treatment | $s_e$ | 0.01 | 0.20 | 0.19 | 0.08 | 0.7 | 5.5 |
| 6B | 4 ♂ | After re- | M | 0.43 | 10.3 | 4.54 | 5.24 | 3.4 | 140.0 |
| | | covering | $s_e$ | 0.01 | 0.30 | 0.19 | 0.39 | 0.6 | 11.0 |
| | | 1 hour | | | | | | | |
| 6C | 4 ♂ | After re- | M | 0.47 | 8.32 | 3.39 | 11.6 | 14.5 | 130.0 |
| | | covering | $s_e$ | 0.11 | 0.80 | 0.36 | 0.88 | 8.0 | 3.5 |
| | | 2 hours | | | | | | | |
| | *Cf* B with A | | *CR* | *+0.2* | *+0.4* | *−0.1* | *−3.2* | *−4.0* | *+1.8* |
| | *Cf* C with A | | *CR* | *+0.6* | *−2.2* | *−2.9* | *+5.8* | *+4.1* | *+2.0* |
| 10A | 6 ♂ | Without | M | 0.53 | 9.13 | 3.82 | 5.37 | 4.0 | 123.0 |
| | N–R | treatment | $s_e$ | 0.05 | 0.30 | 0.19 | 0.39 | 0.6 | 11.0 |
| 10B | 6 ♂ | After re- | M | 0.58 | 9.19 | 3.79 | 3.54 | 2.0 | 130.0 |
| | N–R | covering | $s_e$ | 0.05 | 0.20 | 0.01 | 0.27 | 0.8 | 4.8 |
| | | 1 hour | | | | | | | |
| 10C | 6 ♂ | After re- | M | 0.58 | 8.34 | 3.34 | 10.1 | 7.0 | 142.0 |
| | N–R | covering | $s_e$ | 0.06 | 0.15 | 1.14 | 1.14 | 1.6 | 4.4 |
| | | 2 hours | | | | | | | |
| | *Cf* B with A | | *CR* | *+0.4* | *+0.1* | *−0.1* | *−12.2* | *−2.0* | *+0.2* |
| | *Cf* C with A | | *CR* | *+0.4* | *−3.4* | *−2.8* | *+4.1* | *+1.6* | *+0.8* |

TABLE VII. Blood Values of Normal Breeding and Regressed Males and Females Sampled 2–6 Hours After Capture.

| Exp | Spec | St | GSI | Hgb | RBC | WBC | Eos | Thromb |
|---|---|---|---|---|---|---|---|---|
| 1A | 16 ♂ | M | – | 8.47 | 4.34 | 6.80 | 11.0 | 134.0 |
| | N–B | $s_e$ | – | 0.12 | 0.02 | 0.44 | 1.9 | 6.3 |
| 1B | 16 ♀ | M | – | 6.89 | 3.27 | 6.30 | 15.0 | 122.0 |
| | N–B | $s_e$ | – | 0.12 | 0.05 | 0.36 | 3.2 | 4.3 |
| 5A | 12 ♂ | M | – | 7.19 | 3.54 | 6.70 | 6.0 | 124.0 |
| | N–R | $s_e$ | – | 0.12 | 0.05 | 0.31 | 1.4 | 6.0 |
| 5B | 12 ♀ | M | – | 7.55 | 3.54 | 7.90 | 5.5 | 166.0 |
| | N–R | $s_e$ | – | 0.10 | 0.05 | 0.28 | 1.4 | 6.0 |
| *Cf* | 1B with 1A | *CR* | – | *−9.3* | *−21.0* | *−0.9* | *+1.1* | *−1.6* |
| *Cf* | 5B with 5A | *CR* | – | *+2.4* | *0.0* | *+2.9* | *−0.3* | *+4.7* |
| *Cf* | 5A with 1A | *CR* | – | *−7.5* | *−16.0* | *−0.2* | *−2.2* | *−1.3* |
| *Cf* | 5B with 1B | *CR* | – | *+4.4* | *+13.0* | *+3.5* | *−2.6* | *+5.9* |

TABLE VIII. Blood Values of Fish kept in Aquaria in Either Salt Water or Fresh Water or Both.

Normal Regressed Males and Females kept Either in Salt Water Only or in Fresh Water Only for 3½ Months.

| Exp | Spec | Medium | St | GSI | Hgb | RBC | WBC | Eos | Thromb |
|---|---|---|---|---|---|---|---|---|---|
| 8A | 12 ♂ | Salt | M | 0.52 | 9.03 | 3.93 | 6.05 | 6.0 | 118.0 |
| | N–R | water | $s_e$ | 0.03 | 0.09 | 0.03 | 0.19 | 1.4 | 3.8 |
| 8B | 12 ♀ | Salt | M | 1.23 | 9.22 | 3.94 | 6.90 | 10.0 | 122.0 |
| | N–R | water | $s_e$ | 0.04 | 0.18 | 0.04 | 0.53 | 1.6 | 3.4 |
| 8C | 11 ♂ | Fresh | M | 0.65 | 9.19 | 4.03 | 10.1 | 1.0 | 121.0 |
| | N–R | water | $s_e$ | 0.08 | 0.17 | 0.04 | 0.36 | 0.3 | 3.0 |
| 8D | 13 ♀ | Fresh | M | 1.31 | 9.41 | 4.03 | 9.55 | 3.0 | 124.0 |
| | N–R | water | $s_e$ | 0.09 | 0.18 | 0.01 | 0.34 | 0.7 | 4.4 |
| | *Cf* B with A | | *CR* | *+14.2* | *+0.9* | *+0.2* | *+1.5* | *+1.9* | *+0.8* |
| | *Cf* D with C | | *CR* | *+5.5* | *+0.9* | *0.0* | *—1.1* | *+2.3* | *+0.6* |
| | *Cf* C with A | | *CR* | *+1.5* | *+1.0* | *+2.0* | *+7.4* | *—3.9* | *+0.6* |
| | *Cf* D with B | | *CR* | *+0.8* | *+0.8* | *+2.2* | *+4.2* | *—4.0* | *+0.4* |

Normal Regressed Males and Females, Captured in Autumn, kept in Salt Water Only or in Fresh Water Only until they were Maturing the Following Spring (7 Months). Illumination Eight Hours Daily for Six Months, Seventeen Hours Daily for Last Month.

| Exp | Spec | Medium | St | GSI | Hgb | RBC | WBC | Eos | Thromb |
|---|---|---|---|---|---|---|---|---|---|
| 15A | 14 ♂ | Salt | M | 6.10 | 8.34 | 4.94 | 4.28 | 14.0 | 104.0 |
| | N-M | water | $s_e$ | 0.40 | 0.15 | 0.08 | 0.13 | 1.7 | 3.1 |
| 15B | 14 ♀ | Salt | M | 5.48 | 7.40 | 3.30 | 5.48 | 12.0 | 143.0 |
| | N–M | water | $s_e$ | 1.12 | 0.15 | 0.07 | 0.21 | 1.7 | 4.1 |
| 15C | 10 ♂ | Fresh | M | 4.49 | 7.47 | 4.18 | 6.06 | 11.0 | 120.0 |
| | N–M | water | $s_e$ | 0.39 | 0.12 | 0.12 | 0.19 | 2.0 | 3.3 |
| 15D | 15 ♀ | Fresh | M | 5.05 | 7.18 | 3.08 | 7.07 | 9.0 | 136.0 |
| | N–M | water | $s_e$ | 0.38 | 0.13 | 0.06 | 0.19 | 2.0 | 3.7 |
| | *Cf* B with A | | *CR* | *—0.5* | *—4.5* | *—15.7* | *+4.8* | *—0.9* | *+7.7* |
| | *Cf* D with C | | *CR* | *+0.9* | *—1.6* | *—8.5* | *+3.7* | *—0.9* | *+3.2* |
| | *Cf* C with A | | *CR* | *—2.9* | *—4.6* | *—5.4* | *+7.7* | *—1.5* | *+3.6* |
| | *Cf* D with B | | *CR* | *—0.4* | *—1.2* | *—2.4* | *+5.6* | *—1.5* | *—1.3* |

Normal Breeding Males kept in Salt and Fresh Water: Five Specimens in Salt Water from August 1956–July 1957; Seven Specimens also in Salt Water for the same Period Except for the Last 16 Days, when they were Transferred to Fresh Water.

| Exp | Spec | Medium | St | GSI | Hgb | RBC | WBC | Eos | Thromb |
|---|---|---|---|---|---|---|---|---|---|
| 3 | 5 ♂ | Salt | M | 3.24 | 9.16 | 4.85 | 8.81 | 12.0 | 130.0 |
| | N–B | water | $s_e$ | 0.55 | 0.34 | 0.27 | 0.49 | 1.4 | 4.3 |
| | 7 ♂ | Salt and | M | 3.01 | 9.55 | 4.56 | 9.10 | 11.0 | 143.0 |
| | N–B | fresh water | $s_e$ | 0.11 | 0.23 | 0.29 | 0.51 | 2.7 | 7.7 |
| | | | *CR* | *—0.4* | *+0.9* | *—0.8* | *+0.4* | *—0.3* | *+1.5* |

TABLE IX. Blood Values of Hypophysectomized Males and Females Compared With Those of Normal Breeding and Regressed Specimens, All From Salt Water Only.

| Exp | Spec | St | GSI | Hgb | RBC | WBC | Eos | Thromb |
|---|---|---|---|---|---|---|---|---|
| 8A | 12 ♂ | M | 0.52 | 9.03 | 3.93 | 6.05 | 6.0 | 118.0 |
| | N–R | $s_e$ | 0.03 | 0.09 | 0.03 | 0.19 | 1.4 | 3.8 |
| 19A | 10 ♂ | M | 2.71 | 7.41 | 3.96 | 5.40 | 13.1 | 88.0 |
| | N–B | $s_e$ | 0.25 | 0.25 | 0.08 | 0.47 | 3.1 | 3.6 |
| 2 | 9 ♂ | M | 0.31 | 7.08 | 3.14 | 3.08 | 0 | 127.0 |
| | H | $s_e$ | 0.03 | 0.47 | 0.07 | 0.36 | – | 9.3 |
| 17A | 10 ♂ | M | 0.39 | 5.05 | 2.01 | 1.84 | 1.4 | 125.0 |
| | H | $s_e$ | 0.04 | 0.25 | 0.11 | 0.16 | 0.7 | 3.4 |
| | *Cf* 2 with 8A | *CR* | *—1.2* | *— 4.1* | *—10.0* | *— 7.2* | *—4.3* | *+0.9* |
| | *Cf* 17A with 8A | *CR* | *—2.6* | *—14.8* | *—15.3* | *—16.7* | *—3.4* | *+0.8* |
| | *Cf* 17A with 19A | *CR* | *—9.0* | *—6.7* | *—14.0* | *—7.4* | *—3.7* | *+7.6* |
| 8B | 12 ♀ | M | 1.23 | 9.22 | 3.94 | 6.90 | 10.0 | 122.0 |
| | N–R | $s_e$ | 0.04 | 0.18 | 0.04 | 0.53 | 1.6 | 3.4 |
| 19B | 10 ♀ | M | 3.28 | 6.27 | 2.62 | 4.18 | 6.9 | 83.0 |
| | N–B | $s_e$ | 0.40 | 0.20 | 0.11 | 0.26 | 0.3 | 3.7 |
| 17B | 10 ♀ | M | 0.92 | 6.31 | 2.40 | 1.61 | 1.2 | 81.0 |
| | H | $s_e$ | 0.10 | 0.34 | 0.10 | 0.22 | 0.5 | 3.0 |
| | *Cf* 17B with 8B | *CR* | *—2.8* | *—7.6* | *—14.0* | *—9.5* | *—5.2* | *—8.0* |
| | *Cf* 17B with 19B | *CR* | *—21.5* | *+0.1* | *—2.0* | *—5.1* | *—9.5* | *—0.4* |

TABLE X. Effects of 0.6% NaCl Injections.

Effects on Normal Breeding and Regressed Males and Females Autopsied Two Hours After a Single Injection. All Specimens From Salt Water Only.

| Exp | Spec | Injection | St | GSI | Hgb | RBC | WBC | Eos | Thromb |
|---|---|---|---|---|---|---|---|---|---|
| 10 | 6 ♂ | None | M | 0.53 | 9.13 | 3.82 | 5.37 | 4.0 | 123.0 |
| | N–R | | $s_e$ | 0.05 | 0.30 | 0.19 | 0.39 | 0.6 | 11.0 |
| 11 | 4 ♂ | 0.6% NaCl | M | 0.60 | 9.27 | 3.88 | 4.08 | 8.0 | 132.0 |
| | N–R | | $s_e$ | 0.02 | 0.27 | 0.08 | 0.20 | 0.6 | 9.5 |
| | | | *CR* | *+1.4* | *+0.3* | *+0.5* | *—5.0* | *+3.0* | *+0.8* |
| 14 | 6 ♀ | None | M | 0.75 | 7.55 | 3.13 | 5.73 | 8.0 | 118.0 |
| | N–R | | $s_e$ | 0.16 | 0.15 | 0.03 | 0.17 | 0.6 | 6.0 |
| | 5 ♀ | 0.6% NaCl | M | 0.68 | 9.61 | 3.52 | 4.70 | 6.3 | 116.0 |
| | N–R | | $s_e$ | 0.05 | 0.20 | 0.01 | 0.19 | 1.1 | 6.2 |
| | | | *CR* | *—0.4* | *+8.2* | *+1.3* | *—3.5* | *—1.4* | *—0.2* |

(cont.)

TABLE X. (cont.)

| Exp | Spec | Injection | St | GSI | Hgb | RBC | WBC | Eos | Thromb |
|---|---|---|---|---|---|---|---|---|---|
| 19A | 10 ♂ | None | M | 2.71 | 7.41 | 3.96 | 5.40 | 13.1 | 88.0 |
| | N–B | | $s_e$ | 0.25 | 0.25 | 0.08 | 0.47 | 3.1 | 3.6 |
| 19B | 10 ♂ | 0.6% NaCl | M | 2.38 | 7.08 | 4.18 | 6.41 | 32.0 | 96.0 |
| | N–B | | $s_e$ | 0.31 | 0.15 | 0.15 | 0.30 | 6.0 | 2.2 |
| 19C | 10 ♀ | None | M | 3.28 | 6.27 | 2.62 | 4.18 | 6.9 | 83.0 |
| | N–B | | $s_e$ | 0.40 | 0.20 | 0.11 | 0.26 | 0.3 | 3.7 |
| 19D | 10 ♀ | 0.6% NaCl | M | 2.78 | 6.52 | 2.79 | 3.69 | 32.9 | 87.0 |
| | N–B | | $s_e$ | 0.30 | 0.23 | 0.06 | 0.28 | 4.7 | 3.9 |
| | *Cf* B with A | | *CR* | *−0.8* | *−1.1* | *+1.6* | *+1.8* | *+2.7* | *+1.9* |
| | *Cf* D with C | | *CR* | *−1.0* | *+0.8* | *+1.5* | *−1.3* | *+5.6* | *+0.7* |

Effects on Hypophysectomized Males and Females Autopsied Two Hours After a Single Injection; All Specimens From Salt Water.

| Exp | Spec | Injection | St | GSI | Hgb | RBC | WBC | Eos | Thromb |
|---|---|---|---|---|---|---|---|---|---|
| 2 | 9 ♂ | None | M | 0.31 | 7.08 | 3.14 | 3.08 | 0 | 127.0 |
| | H | | $s_e$ | 0.03 | 0.47 | 0.07 | 0.36 | – | 9.3 |
| 4 | 4 ♂ | 0.6% NaCl | M | 0.29 | 8.85 | 3.61 | 5.38 | 7.0 | 102.0 |
| | H | | $s_e$ | 0.04 | 0.33 | 0.18 | 0.37 | 1.9 | 4.2 |
| 7 | 5 ♂ | 0.6% NaCl | M | 0.24 | 7.09 | 3.00 | 5.08 | 3.0 | 86.0 |
| | H | | $s_e$ | 0.02 | 0.21 | 0.06 | 0.22 | 1.1 | 5.5 |
| 4+7 | 9 ♂ | 0.6% NaCl | M | 0.26 | 7.87 | 3.27 | 5.21 | 5.0 | 93.0 |
| | H | | $s_e$ | 0.03 | 0.31 | 0.14 | 0.23 | 1.3 | 4.7 |
| | *Cf* 4 with 2 | | *CR* | *−0.4* | *+1.4* | *+2.5* | *+4.4* | *+4.0* | *−2.4* |
| | *Cf* 7 with 2 | | *CR* | *−1.7* | *+0.2* | *−1.6* | *+4.8* | *+3.0* | *−3.8* |
| | *Cf* 4+7 with 2 | | *CR* | *−1.7* | *+1.4* | *+0.8* | *+5.1* | *+4.0* | *−3.1* |
| 17A | 10 ♂ | None | M | 0.39 | 5.05 | 2.01 | 1.84 | 1.4 | 125.0 |
| | H | | $s_e$ | 0.04 | 0.25 | 0.11 | 0.16 | 0.7 | 3.4 |
| 17B | 10 ♂ | 0.6% NaCl | M | 0.49 | 5.88 | 2.29 | 2.17 | 0.3 | 107.0 |
| | H | | $s_e$ | 0.04 | 0.19 | 0.09 | 0.15 | – | 4.3 |
| 17C | 10 ♀ | None | M | 0.92 | 6.31 | 2.40 | 1.61 | 1.2 | 81.0 |
| | H | | $s_e$ | 0.10 | 0.34 | 0.10 | 0.22 | 0.5 | 3.0 |
| 17D | 10 ♀ | 0.6% NaCl | M | 1.01 | 5.86 | 2.24 | 2.17 | 2.4 | 95.0 |
| | H | | $s_e$ | 0.05 | 0.23 | 0.14 | 0.22 | 0.9 | 4.8 |
| | *Cf* B with A | | *CR* | *+1.7* | *+2.7* | *+2.0* | *+1.5* | – | *−3.2* |
| | *Cf* D with C | | *CR* | *+0.8* | *−1.1* | *−0.9* | *+1.7* | *+1.2* | *+2.3* |

TABLE XI. EFFECTS OF ACTH.

Effects on Normal Males and Females, Regressed and Breeding, by Comparison with Specimens Injected with 0.6% NaCl; Autopsied Two Hours After a Single Injection.

| Exp | Spec | Injection | St | GSI | Hgb | RBC | WBC | Eos | Thromb |
|---|---|---|---|---|---|---|---|---|---|
| 4 | 6 ♂ | 0.6% NaCl | M | 1.69 | 10.3 | 4.58 | 6.13 | 8.0 | 168.0 |
| | N–R | | $s_e$ | 0.45 | 0.14 | 0.16 | 0.31 | 0.4 | 6.7 |
| | 4 ♂ | ACTH | M | 0.53 | 9.30 | 4.16 | 30.5 | 17.0 | 197.0 |
| | N–R | (Prep. 1b) | $s_e$ | 0.13 | 0.31 | 0.13 | 0.39 | 3.1 | 4.2 |
| | | | *CR* | *−2.5* | *−3.0* | *−2.0* | *+6.4* | *+2.9* | *+3.7* |
| 11 | 4 ♂ | 0.6% NaCl | M | 0.60 | 9.27 | 3.88 | 4.08 | 8.0 | 132.0 |
| | N–R | | $s_e$ | 0.02 | 0.27 | 0.08 | 0.20 | 0.6 | 9.5 |
| | 6 ♂ | ACTH | M | 0.43 | 8.41 | 3.43 | 24.1 | 18.0 | 108.0 |
| | N–R | (Prep. 1c) | $s_e$ | 0.06 | 0.18 | 0.10 | 0.12 | 3.4 | 5.6 |
| | | | *CR* | *−2.8* | *−2.7* | *−5.8* | *+15.0* | *+3.0* | *−2.2* |
| 19A | 10 ♂ | 0.6% NaCl | M | 2.38 | 7.08 | 4.18 | 6.41 | 32.0 | 96.0 |
| | N–B | | $s_e$ | 0.31 | 0.15 | 0.15 | 0.30 | 6.0 | 2.2 |
| 19B | 10 ♂ | ACTH | M | 2.73 | 6.37 | 3.58 | 26.0 | 28.1 | 144.0 |
| | N–B | (Prep. 1c) | $s_e$ | 0.21 | 0.27 | 0.14 | 1.88 | 7.0 | 5.6 |
| 19C | 10 ♀ | 0.6% NaCl | M | 2.78 | 6.52 | 2.79 | 3.69 | 32.9 | 87.0 |
| | N–B | | $s_e$ | 0.30 | 0.23 | 0.06 | 0.28 | 4.7 | 3.9 |
| 19D | 10 ♀ | ACTH | M | 3.69 | 6.71 | 2.91 | 29.87 | 35.3 | 105.0 |
| | N–B | (Prep. 1c) | $s_e$ | 0.78 | 0.21 | 0.09 | 1.89 | 8.0 | 2.7 |
| *Cf* | B with A | | *CR* | *+0.9* | *−2.3* | *−2.9* | *+2.9* | *−0.5* | *+6.7* |
| *Cf* | D with C | | *CR* | *+1.1* | *+0.6* | *+1.1* | *+4.3* | *+0.9* | *+3.6* |

Effects on Hypophysectomized Males and Females by Comparison with Specimens Injected with 0.6% NaCl; Autopsied Two Hours After a Single Injection.

| Exp | Spec | Injection | St | GSI | Hgb | RBC | WBC | Eos | Thromb |
|---|---|---|---|---|---|---|---|---|---|
| 4 | 4 ♂ | 0.6% NaCl | M | 0.29 | 8.85 | 3.61 | 5.38 | 7.0 | 102.0 |
| | H | | $s_e$ | 0.04 | 0.33 | 0.18 | 0.37 | 1.9 | 4.2 |
| | 4 ♂ | ACTH | M | 0.22 | 6.75 | 2.87 | 11.90 | 2.0 | 125.0 |
| | H | (Prep. 1b) | $s_e$ | 0.02 | 0.38 | 0.33 | 0.81 | 0.5 | 2.5 |
| | | | *CR* | *−1.6* | *−4.0* | *−2.0* | *+8.2* | *−2.4* | *+4.8* |
| 17A | 10 ♂ | 0.6% NaCl | M | 0.49 | 5.88 | 2.29 | 2.17 | 0.3 | 107.0 |
| | H | | $s_e$ | 0.04 | 0.19 | 0.09 | 0.15 | – | 4.3 |
| 17B | 10 ♂ | ACTH | M | 0.41 | 6.05 | 2.25 | 22.16 | 15.4 | 102.0 |
| | H | (Prep. 1c) | $s_e$ | 0.05 | 0.21 | 0.06 | 2.01 | 5.0 | 3.6 |
| 17C | 10 ♀ | 0.6% NaCl | M | 1.01 | 5.86 | 2.24 | 2.17 | 2.4 | 95.0 |
| | H | | $s_e$ | 0.05 | 0.23 | 0.14 | 0.22 | 0.9 | 4.8 |
| 17D | 10 ♀ | ACTH | M | 1.53 | 6.73 | 2.64 | 41.55 | 30.7 | 69.0 |
| | H | (Prep. 1c) | $s_e$ | 0.33 | 0.33 | 0.16 | 3.97 | 8.0 | 4.6 |
| *Cf* | B with A | | *CR* | *−1.3* | *+0.6* | *−0.3* | *+9.1* | + Sig. | *−1.0* |
| *Cf* | D with C | | *CR* | *+1.6* | *+2.2* | *+1.9* | *+9.8* | *+3.2* | *−5.3* |

(cont.)

TABLE XI. (cont.)

Effects on Hypophysectomized Males That Were Injected Thrice Weekly for One Month and Autopsied Twenty-Four Hours After Final Injection, Compared With Uninjected Males.

| Exp | Spec | Injection | St | GSI | Hgb | RBC | WBC | Eos | Thromb |
|---|---|---|---|---|---|---|---|---|---|
| 2 | 9 ♂ | None | M | 0.31 | 7.08 | 3.14 | 3.08 | 0 | 127.0 |
| | H | | $s_e$ | 0.03 | 0.47 | 0.07 | 0.36 | – | 9.3 |
| | 10 ♂ | $\alpha_8$-ACTH | M | 0.34 | 9.86 | 4.45 | 7.10 | 4.4 | 121.0 |
| | H | (Prep. 1a) | $s_e$ | 0.01 | 0.06 | 0.32 | 0.43 | 1.1 | 4.5 |
| | | | *CR* | *+1.0* | *+5.9* | *+4.0* | *+7.1* | *+4.0* | *—0.6* |

TABLE XII. EFFECTS OF CORTISOL INJECTIONS.

Effects on Normal Regressed, Breeding and Maturing Males and on Normal Regressed and Maturing Females Autopsied Two Hours After a Single Injection.

| Exp | Spec | Injection | St | GSI | Hgb | RBC | WBC | Eos | Thromb |
|---|---|---|---|---|---|---|---|---|---|
| 7 | 4 ♂ | 0.6% NaCl | M | 0.49 | 7.75 | 3.68 | 5.13 | 2.5 | 107.0 |
| | N–R | | $s_e$ | 0.02 | 0.34 | 0.18 | 0.23 | 0.8 | 4.2 |
| | 4 ♂ | Cortisol | M | 0.38 | 8.63 | 4.46 | 3.44 | 0.8 | 110.0 |
| | N–R | | $s_e$ | 0.05 | 0.53 | 0.17 | 0.27 | 0.4 | 7.0 |
| | | | *CR* | *—0.5* | *+1.4* | *+3.1* | *—5.1* | *—2.1* | *+0.4* |
| 19 | 10 ♂ | 0.6% NaCl | M | 2.38 | 7.08 | 4.18 | 6.41 | 32.0 | 96.0 |
| | N–B | | $s_e$ | 0.31 | 0.15 | 0.15 | 0.30 | 6.0 | 2.2 |
| | 10 ♂ | Cortisol | M | 2.74 | 6.45 | 3.69 | 15.56 | 47.8 | 92.0 |
| | N–B | | $s_e$ | 0.23 | 0.27 | 0.12 | 1.03 | 7.0 | 3.3 |
| | | | *CR* | *+0.9* | *—2.0* | *—2.6* | *+8.8* | *+2.0* | *—1.1* |
| 14 | 5 ♀ | 0.6% NaCl | M | 0.68 | 9.61 | 3.52 | 4.70 | 6.3 | 116.0 |
| | N–R | | $s_e$ | 0.05 | 0.20 | 0.01 | 0.19 | 1.1 | 6.2 |
| | 6 ♀ | Cortisol | M | 0.78 | 8.40 | 3.58 | 2.98 | 0.5 | 85.0 |
| | N–R | | $s_e$ | 0.10 | 0.33 | 0.13 | 0.22 | 0.2 | 4.0 |
| | | | *CR* | *+0.9* | *—3.2* | *+0.5* | *—6.0* | *—5.3* | *—4.3* |
| 16* | 5 ♂ | 0.6% NaCl | M | 1.82 | 6.59 | 4.42 | 8.38 | 11.7 | 112.0 |
| | N–M | | $s_e$ | 0.32 | 0.53 | 0.26 | 0.12 | 3.2 | 7.3 |
| | 6 ♂ | Cortisol | M | 3.44 | 7.64 | 4.74 | 9.28 | 28.4 | 100.0 |
| | N–M | | $s_e$ | 2.11 | 0.27 | 0.07 | – | 6.7 | 5.2 |
| | 2 ♀ | None | M | 9.59 | 6.94 | 2.97 | 4.30 | 5.0 | 120.0 |
| | N–M | | $s_e$ | 2.67 | 0.17 | 0.03 | 0.28 | 0.4 | 0.0 |
| | 10 ♀ | 0.6% NaCl | M | – | 7.03 | 2.94 | 5.00 | 34.3 | 113.0 |
| | N–M | | $s_e$ | – | 0.23 | 0.03 | 0.28 | 6.9 | 7.7 |
| | 10 ♀ | Cortisol | M | – | 5.95 | 2.78 | 6.84 | 25.6 | 112.0 |
| | N–M | | $s_e$ | – | 0.35 | 0.04 | – | 6.6 | 4.3 |

* Critical ratios intentionally omitted. See list of data for each individual specimen at end of this table.

(cont.)

TABLE XII. (cont.)

Effects on Hypophysectomized Males and Females Autopsied Two Hours after a Single Injection.

| Exp | Spec | Injection | St | GSI | Hgb | RBC | WBC | Eos | Thromb |
|---|---|---|---|---|---|---|---|---|---|
| 7 | 5 ♂ | 0.6% NaCl | M | 0.24 | 7.09 | 3.00 | 5.08 | 3.0 | 86.0 |
| | H | | $s_e$ | 0.02 | 0.21 | 0.06 | 0.22 | 1.1 | 5.5 |
| | 5 ♂ | Cortisol | M | 0.36 | 6.46 | 3.01 | 15.70 | 16.0 | 124.0 |
| | H | | $s_e$ | 0.03 | 0.07 | 0.33 | 0.78 | 0.7 | 7.0 |
| | | | *CR* | *+3.3* | *−2.9* | *0* | *+13.1* | *+10.0* | *+4.3* |
| 17A | 10 ♂ | 0.6% NaCl | M | 0.49 | 5.88 | 2.29 | 2.17 | 0.3 | 107.0 |
| | H | | $s_e$ | 0.04 | 0.19 | 0.09 | 0.15 | – | 4.3 |
| 17B | 10 ♂ | Cortisol | M | 0.46 | 5.37 | 1.92 | 9.04 | 8.2 | 200.0 |
| | H | | $s_e$ | 0.04 | 0.30 | 0.13 | 0.58 | 2.9 | 5.4 |
| 17C | 10 ♀ | 0.6% NaCl | M | 1.01 | 5.86 | 2.24 | 2.17 | 2.4 | 95.0 |
| | H | | $s_e$ | 0.05 | 0.23 | 0.14 | 0.22 | 0.9 | 4.8 |
| 17D | 10 ♀ | Cortisol | M | 1.58 | 6.06 | 2.21 | 21.97 | 31.2 | 152.0 |
| | H | | $s_e$ | 0.68 | 0.29 | 0.15 | 1.83 | 4.0 | 5.2 |
| | *Cf* B with A | | *CR* | *−0.6* | *−2.2* | *−2.3* | *+36.0* | +sig. | *+13.6* |
| | *Cf* D with C | | *CR* | *+0.8* | *+0.9* | *−0.1* | *+10.1* | *+7.1* | *+8.2* |

| Exp | Spec | Injection | GSI | Hgb | RBC | WBC | Eos | Thromb |
|---|---|---|---|---|---|---|---|---|
| 16 | 376 | 0.6% NaCl | 1.30 | 6.35 | 4.11 | 7.50 | 36 | 110.0 |
| ♂♂ | 377 | ,, | 2.17 | 7.45 | 5.03 | 7.85 | 9 | 130.0 |
| N–M | 378 | ,, | 1.34 | 7.86 | 5.19 | 9.80 | 36 | 130.0 |
| | 379 | ,, | 1.23 | 5.52 | 3.77 | 8.25 | 20 | 90.0 |
| | 380 | ,, | 3.08 | 5.79 | 4.00 | 8.50 | 16 | 100.0 |
| | 381 | Cortisol | 3.51 | 8.70 | 5.01 | 12.20 | 34 | 90.0 |
| | 382 | ,, | 3.53 | 7.59 | 4.89 | 9.95 | 52 | 100.0 |
| | 383 | ,, | 2.05 | 6.90 | 4.55 | 8.50 | 52 | 100.0 |
| | 384 | ,, | 5.18 | 7.09 | 4.74 | 15.00 | 40 | 90.0 |
| | 385 | ,, | 1.23 | 7.74 | 4.66 | 5.15 | 30 | 110.0 |
| | 386 | ,, | 1.70 | 7.86 | 4.58 | 4.85 | 10 | 110.0 |
| 16 | 374 | No Inject. | 5.86 | 7.45 | 3.08 | 4.50 | 12 | 120.0 |
| ♀♀ | 375 | ,, | 13.32 | 6.43 | 2.75 | 4.15 | 36 | 120.0 |
| N–M | 354 | 0.6% NaCl | 2.01 | 6.80 | 2.87 | 4.55 | 31 | 100.0 |
| | 355 | ,, | 1.78 | 5.78 | 2.61 | 4.70 | 34 | 120.0 |
| | 356 | ,, | 8.25 | 7.68 | 2.85 | 4.15 | 8 | 110.0 |
| | 357 | ,, | 3.71 | 7.45 | 3.11 | 5.25 | 30 | 120.0 |
| | 358 | ,, | 5.91 | 6.21 | 2.92 | 4.55 | 52 | 100.0 |
| | 359 | ,, | 3.80 | 7.32 | 2.96 | 5.00 | 46 | 130.0 |
| | 360 | ,, | 4.07 | 8.14 | 3.20 | 5.85 | 43 | 130.0 |
| | 361 | ,, | 2.29 | 6.35 | 2.71 | 5.60 | 37 | 110.0 |
| | 362 | ,, | 1.18 | 8.43 | 3.29 | 5.85 | 26 | 110.0 |
| | 363 | ,, | 1.79 | 6.21 | 2.88 | 4.50 | 30 | 100.0 |

(cont.)

TABLE XII. (cont.)

| Exp | Spec | Injection | GSI | Hgb | RBC | WBC | Eos | Thromb |
|---|---|---|---|---|---|---|---|---|
| | 364 | Cortisol | 5.89 | 5.79 | 2.93 | 12.55 | 40 | 100.0 |
| | 365 | ,, | 1.29 | 5.52 | 2.69 | 4.10 | 27 | 110.0 |
| | 366 | ,, | 17.09 | 5.93 | 2.78 | 13.35 | 38 | 100.0 |
| | 367 | ,, | 4.61 | 6.21 | 3.01 | 9.75 | 28 | 100.0 |
| | 368 | ,, | 2.27 | 5.93 | 2.66 | 4.95 | 32 | 110.0 |
| | 369 | ,, | 3.09 | 5.79 | 2.84 | 8.20 | 8 | 110.0 |
| | 370 | ,, | 1.50 | 5.65 | 2.77 | 3.00 | 14 | 100.0 |
| | 371 | ,, | 3.48 | 5.57 | 2.67 | 3.50 | 24 | 90.0 |
| | 372 | ,, | 1.93 | 6.08 | 2.55 | 2.00 | 22 | 110.0 |
| | 373 | ,, | 5.47 | 6.76 | 2.88 | 7.00 | 23 | 110.0 |

TABLE XIII. Effects of Prolactin, ACTH, and Intermedin on Hypophysectomized Males That Were Injected Thrice Weekly for One Month and Autopsied 24 Hours After Last Injection.

| Exp | Spec | Injection | St | GSI | Hgb | RBC | WBC | Eos | Thromb |
|---|---|---|---|---|---|---|---|---|---|
| 2A | 9 ♂ | None | M | 0.31 | 7.08 | 3.14 | 3.08 | 0 | 127.0 |
| | H | | $s_e$ | 0.03 | 0.47 | 0.07 | 0.36 | – | 9.3 |
| 2B | 10 ♂ | Prol. | M | 0.34 | 7.40 | 3.26 | 2.45 | 6.0 | 111.0 |
| | H | | $s_e$ | 0.02 | 0.33 | 0.14 | 0.23 | 1.5 | 3.9 |
| 2C | 10 ♂ | $\alpha_s$-ACTH | M | 0.34 | 9.86 | 4.45 | 7.10 | 4.4 | 121.0 |
| | H | (Prep. 1a) | $s_e$ | 0.01 | 0.06 | 0.32 | 0.43 | 1.1 | 4.5 |
| 2D | 10 ♂ | Prol. + | M | 0.44 | 8.79 | 3.92 | 5.07 | 5.0 | 143.0 |
| | H | $\alpha_s$-ACTH (Prep. 1a) | $s_e$ | 0.04 | 0.31 | 0.15 | 0.48 | 1.6 | 6.8 |
| | *Cf* B with A | | *CR* | *+1.0* | *+0.7* | *+0.3* | *−1.5* | *+6.0* | *−1.6* |
| | *Cf* C with A | | *CR* | *+1.0* | *+5.9* | *+4.0* | *+7.1* | *+4.0* | *−0.6* |
| | *Cf* D with A | | *CR* | *+2.6* | *+3.1* | *+4.6* | *+3.3* | *+5.0* | *+1.4* |
| 9A | 10 ♂ | 0.6% NaCl | M | 0.26 | 5.99 | 2.71 | 4.34 | 0 | 101.0 |
| | H | | $s_e$ | 0.02 | 0.08 | 0.51 | 0.39 | – | 4.4 |
| 9B | 10 ♂ | Prol. | M | 0.23 | 6.76 | 2.84 | 7.31 | 8.1 | 129.0 |
| | H | | $s_e$ | 0.01 | 0.22 | 0.14 | 0.49 | 1.5 | 5.0 |
| 9C | 10 ♂ | Interm. | M | 0.28 | 5.07 | 2.51 | 5.65 | 0 | 122.0 |
| | H | | $s_e$ | 0.03 | 0.05 | 0.37 | 0.43 | – | 6.5 |
| 9D | 10 ♂ | Prol. + | M | 0.31 | 7.33 | 3.26 | 5.73 | 0 | 115.0 |
| | H | Interm. | $s_e$ | 0.02 | 0.13 | 0.44 | 0.27 | – | 5.2 |
| | *Cf* B with A | | *CR* | *−1.5* | *+3.4* | *+0.25* | *+4.8* | *+8.1* | *+4.2* |
| | *Cf* C with A | | *CR* | *+0.6* | *−10.0* | *−0.3* | *+2.3* | – | *+2.7* |
| | *Cf* D with A | | *CR* | *+1.1* | *+8.4* | *+0.67* | *+3.0* | – | *+2.0* |

TABLE XIV. EFFECTS OF THYROTROPIN (TSH) AND METHYL-TESTOSTERONE ON HYPOPHYSECTOMIZED MALES INJECTED THRICE WEEKLY FOR ONE MONTH AND AUTOPSIED 24 HOURS AFTER THE FINAL INJECTION.

| Exp | Spec | Injection | St | GSI | Hgb | RBC | WBC | Eos | Thromb |
|---|---|---|---|---|---|---|---|---|---|
| 20A | 5 ♂ | 0.6% NaCl | M | 0.26 | 5.26 | 2.51 | 2.51 | 0 | 92.0 |
| | H | | $s_e$ | 0.01 | 0.18 | 0.19 | 0.18 | – | 3.4 |
| 20B | 8 ♂ | TSH | M | 0.49 | 6.06 | 3.19 | 2.86 | 1.7 | 116.0 |
| | H | | $s_e$ | 0.05 | 0.28 | 0.21 | 0.14 | 2.0 | 4.1 |
| 20C | 8 ♂ | Methyl- | M | 1.02 | 5.97 | 3.79 | 2.88 | 0.6 | 123.0 |
| | H | test. | $s_e$ | 0.05 | 0.28 | 0.09 | 0.17 | 0.5 | 4.3 |
| | *Cf* B with A | | *CR* | *+4.6* | *+2.4* | *+2.5* | *+1.5* | *+0.9* | *+4.5* |
| | *Cf* C with A | | *CR* | *+15.2* | *+2.1* | *+6.1* | *+1.4* | *+1.1* | *+6.1* |

TABLE XV. EFFECTS OF HYPOTHYROIDISM ON NORMAL BREEDING MALES KEPT IN SALT WATER (3A, 3B) OR FRESH WATER (3C, 3D). THE HYPOTHYROID SPECIMENS WERE TREATED WITH $I^{131}$

| Exp | Spec | Treatment | St | GSI | Hgb | RBC | WBC | Eos | Thromb |
|---|---|---|---|---|---|---|---|---|---|
| 3A | 5 ♂ | None | M | 3.24 | 9.16 | 4.85 | 8.81 | 12.0 | 130.0 |
| | N–B | Salt water | $s_e$ | 0.55 | 0.34 | 0.27 | 0.49 | 1.4 | 4.3 |
| 3B | 7 ♂ | $I^{131}$ | M | 3.09 | 8.98 | 4.32 | 5.54 | 9.0 | 127.0 |
| | N–B | Salt water | $s_e$ | 0.64 | 0.37 | 0.23 | 0.70 | 0.9 | 6.9 |
| 3C | 7 ♂ | None | M | 3.01 | 9.55 | 4.56 | 9.10 | 11.0 | 143.0 |
| | N–B | Fresh water | $s_e$ | 0.11 | 0.23 | 0.29 | 0.51 | 2.7 | 7.7 |
| 3D | 9 ♂ | $I^{131}$ | M | 2.12 | 8.18 | 4.03 | 8.99 | 8.0 | 124.0 |
| | N–B | Fresh water | $s_e$ | 0.50 | 0.32 | 0.14 | 0.63 | 1.5 | 5.3 |
| | *Cf* B with A | | *CR* | *−0.2* | *−0.4* | *−1.5* | *−6.4* | *−1.9* | *−0.5* |
| | *Cf* D with C | | *CR* | *−1.7* | *−3.6* | *−1.6* | *−0.1* | *−1.0* | *−2.0* |
| | *Cf* C with A | | *CR* | *−0.4* | *+0.9* | *−0.8* | *+0.4* | *−0.3* | *+1.5* |
| | *Cf* D with B | | *CR* | *−1.2* | *−1.7* | *−1.1* | *+3.7* | *−0.7* | *−0.4* |

TABLE XVI. RESULTS FROM SALINE TWO HOURS AFTER A SINGLE INJECTION.

| Blood | Exp. | Normal | | Exp. | Hypophysectomized | |
|---|---|---|---|---|---|---|
| | | Males | Females | | Males | Females |
| WINTER | | | | | | |
| Hgb | ♂: 10, 11 | incr. | INCR. | | | |
| RBC | ♀: 14 | incr. | incr. | | | |
| WBC | | DECR. | DECR. | | | |
| Eos | | INCR. | decr. | | | |
| Thromb. | | incr. | decr. | | | |
| SUMMER | | | | | | |
| Hgb | 19 | decr. | incr. | 17 | INCR. | decr. |
| RBC | | incr. | incr. | | INCR. | decr. |
| WBC | | incr. | decr. | | incr. | incr. |
| Eos | | INCR. | INCR. | | decr. | incr. |
| Thromb | | incr. | incr. | | DECR. | INCR. |

incr. = increase not significant.
INCR. = increase significant.
decr. = decrease not significant.
DECR. = decrease significant.

TABLE XVII. RESULTS FROM ACTH TWO HOURS AFTER A SINGLE INJECTION.

| Blood | Exp. | Normal | | Exp. | Hypophysectomized | |
|---|---|---|---|---|---|---|
| | | Males | Females | | Males | Females |
| WINTER | | | | | | |
| Hgb | 4 | DEC . | | 4 | DECR. | |
| RBC | | DECR. | | | DECR. | |
| WBC | | INCR. (5×) | | | INCR. (2×) | |
| Eos | | INCR. | | | DECR. | |
| Thromb. | | INCR. | | | INCR. | |
| Hgb | 11 | DECR. | | | | |
| RBC | | DECR. | | | | |
| WBC | | INCR. (6×) | | | | |
| Eos | | INCR. | | | | |
| Thromb. | | DECR. | | | | |
| SUMMER | | | | | | |
| Hgb | 19 | DECR. | incr. | 17 | incr. | INCR. |
| RBC | | DECR. | incr. | | decr. | incr. |
| WBC | | INCR. (4×) | INCR. (10×) | | INCR. (10×) | INCR. (20×) |
| Eos | | decr. | incr. | | INCR. | INCR. |
| Thromb. | | INCR. | INCR. | | decr. | DECR. |

TABLE XVIII. RESULTS OF CORTISOL TWO HOURS AFTER A SINGLE INJECTION.

| Blood | Exp. | Normal | | Exp. | Hypophysectomized | |
|---|---|---|---|---|---|---|
| | | Males | Females | | Males | Females |
| WINTER | | | | | | |
| Hgb | ♂: 7 | incr. | DECR. | 7 | DECR. | |
| RBC | ♀: 14 | INCR. | incr. | | No change | |
| WBC | | DECR. | DECR. | | INCR. (3×) | |
| Eos | | DECR. | DECR. | | INCR. | |
| Thromb. | | incr. | DECR. | | INCR. | |
| SUMMER | | | | | | |
| Hgb | 19 | DECR. | | 17 | DECR. | incr. |
| RBC | | DECR. | | | DECR. | decr. |
| WBC | | INCR. (2½×) | | | INCR. (4×) | INCR. (10×) |
| Eos | | INCR. | | | INCR. | INCR. |
| Thromb. | | decr. | | | INCR. | INCR. |

## DISCUSSION

### OBSERVATIONS ON NORMAL FISH

*Hemoglobin and Red Blood Cells.* In sexually mature mammals the total red cell count of the male is higher than that of the female, this difference being attributed to the influence of the sex hormones. Sex differences are also well known in fishes (Lange, 1919; Schlicher, 1927; Kalashnikov, 1939a; Puchkov, 1954; Gelineo, 1958; Drabkina, 1958; Molnar, *et al.*, 1959), but the matter is complicated by the annual seasonal sexual cycle. In the majority of species which have been investigated there is a fall in the red cell count during hibernation (Schaefer, 1925; Schlicher, 1927; Katz, 1949), but this decline has been attributed for the most part to factors other than the influence of sex hormones. It is undoubtedly true that dietary deficiencies can result in anemia in fishes (Drabkina, 1951; Halver, 1953; Phillips and Brockway, 1957), and many authors have attributed the anemia of hibernation to lack of food (Hoffmeyer, 1907; Brunner, *et al.*, 1958; Murachi, 1959). However, Schaefer (1925) showed that in the pumpkinseed (*Eupomotis gibbosus*) the red cell count returned to normal in the spring, even during continued starvation, although he attributed the winter anemic condition to a deficient diet. Vysheslavtzeva (1956), who found a seasonal decrease in the hemoglobin of the wild carp (sazan) of the Volga Delta, has noted that hemoglobin in the blood of males was greater than that of females and, contrary to what is found in other fishes, this difference persisted during winter. On the other hand, in *Tilapia*

*macrocephala*, which breeds in aquaria throughout the whole year, Slicher (1951) found a sex difference in both hemoglobin and in the red cell count but observed no seasonal changes. Other investigators (*e.g.*, Spoor, 1951) have studied the influence of temperature, and Katz (1949) and Katz and Southward (1950) believed that the drop in the number of red cells in *Oncorhynchus kisutch* during winter could be due to the lowering of environmental temperature. Oxygen deficiency, discussed below, is important, and many other environmental factors may be concerned in the regulation of the blood picture (*e.g.*, pH; Kalashnikov, 1939b). In order to clarify the role of sex hormones it is therefore imperative to employ experimental procedures that will exclude these collateral factors.

One aspect of the present investigation was directed toward these problems (Slicher, 1958b). During the breeding season, in June, the hemoglobin and red cell counts of wild male *F. heteroclitus* were significantly greater than those of wild females. On the other hand, in sexually regressed fish, taken in November at the onset of winter hibernation, these values were alike in the two sexes (Fig. 1); the red cell count decreased in males by 18.4% and increased in females by 8.3% to a common intermediate value. Hemoglobin values levelled off in a similar manner but were slightly higher in females than in males.

From these experiments it is clear that there is a considerable difference in the red cell count of breeding males and females and that, unlike the Volga sazan, this difference disappears during sexual regression. The role of temperature was excluded by the observation that laboratory fish maintained at 20°C pass into sexual regression during the winter months and show changes in the hematological picture similar to those observed in wild fish. The sex difference disappeared in winter and reappeared in the spring, under conditions of constant temperature. Experiments with male sex hormone, discussed below, confirm the importance of androgens in stimulating the red cell count.

The effects of adaptation to a fresh water environment are difficult to interpret: in winter there was a trend to higher hemoglobin and red cell counts in fish kept in fresh water whereas at the time of sexual maturation the response was reversed. This might indicate greater metabolic needs, at this season, in salt water. In the west coast species, *Fundulus parvipennis*, Keys (1931) found that transfer to fresh water was accompanied by a transitory increase in respiration but that this later returned to normal levels. On the other hand, the experiments of Hickman (1959) on the starry flounder, *Platichthys stellatus*, showed that the standard metabolic rate is significantly less in fresh than in salt water.

Although, as noted above, parallel changes were observed in wild and laboratory-kept fishes, the absolute values for hemoglobin and red cell counts were higher in the latter group in both summer and winter. The reason for this could not be determined. It was thought that wild fish brought to the laboratory for hematological study might be in a state of shock and that this might result in a fall in the red cell count, as seen in the case of cold shock

(see below). A field experiment was performed to elucidate this problem: the red cell count taken in the laboratory four hours after capture showed a slight but statistically insignificant drop as compared with samples taken in the field within a few minutes of capture, but it cannot wholly account for the phenomenon.

Various environmental factors were considered. Although wild fish appear to be well fed, the diet in nature is different from that in the laboratory. As noted above, anemia in fishes has been attributed to a deficient diet and the well balanced laboratory formula, certainly not deficient in vitamins, may have replaced these or other unknown deficiencies in the natural food. This problem was not subjected to experimental investigation. Temperature is apparently excluded, since wild fish were taken at low temperatures in winter and at warm temperatures in summer while laboratory specimens were kept at a fairly constant temperature. Yet the difference persisted at all seasons. Wild fish are subjected to constant changes in salinity, which varies with the tides and movements of the fish. The red cell count of laboratory-kept fish in fresh water was higher in winter but lower at the approach of the breeding season, and it seems unlikely that salinity changes can account for the consistently lower values in wild fish at all seasons. It is known that a deficiency of oxygen will produce an increase in the red cell count of fishes (Hall, *et al.*, 1926; Phillips, 1947). The oxygen content of the aquarium water varied from 85 to 95% of saturation and, if there were a partial deficiency of oxygen in the turbid water of the natural habitat one might suppose that fish taken from this environment would show a higher red cell count than that of aquarium-kept fish, but this is not so. If the natural habitat is well oxygenated then this factor should in any case be excluded.

The greater activity of wild fish may also be a contributing factor. Greater activity, at similar partial pressures of oxygen, would promote a greater per-minute rate of flow of water over the gills and would therefore provide a greater supply of oxygen to the blood. Under these circumstances the red cell count might be lower. The short term experiments of Black (1951), in which an increase in the number of erythrocytes was observed after two to three hours of forced activity, appear to reflect a specific stress reaction and throw no light on possible chronic adaptation to a quiescent habitat.

*White Blood Cells.* Sexual differences in the white cell count were observed in wild fish, but the results are difficult to interpret. During the breeding season the white cell count was higher in males than in females, but in winter regression the reverse was true. In laboratory fish there was no significant difference in the white cell count of males and females during sexual regression, and these values were higher than those in wild fish at this season of the year. At the onset of the breeding season in laboratory-kept fish, a sexual difference appeared, but the females had a higher count, contrary to what was observed in wild

fish. It was noted by Schlicher (1927) that an increase in temperature was followed by an increase in the total white cell count in several freshwater teleosts. This possibility might be taken into consideration when comparing wild fish with laboratory ones, since the latter (at 20°C) showed higher values than wild fish during winter months. On the other hand, during the summer, laboratory fish kept at a constant temperature showed lower white cell counts than those of wild fish, the latter presumably being subjected to fluctuating high temperatures. This difference was noted in both sexes.

Some investigators (Heesen, 1924; Schaefer, 1925) have noted that the white cell count is higher during activity, but since laboratory fish had higher counts in winter and lower counts in summer, compared with wild fish, this explanation cannot be employed.

The effects of salinity on the white cell count appear to be important. Laboratory experiments at constant temperature showed that the white cell counts in both sexes are higher in fresh water than in salt water at all seasons. As with the red cell count, a chronic state of slight stress in this predominantly brackish water species might account for an increase in the white cell count in fresh water.

*Eosinophils.* No statistical significant sexual differences in the eosinophil count of wild fish were observed either in winter or summer. The same was true of laboratory-kept fish, with the exception of regressed females in fresh water, which had a significantly higher count than males. On the other hand, the eosinophil count of wild fish was 50% lower in winter than in summer; this difference was also observed in the laboratory, with the exception of females in salt water, which showed little change in summer.

The laboratory experiments with fresh and salt water gave interesting results. After three and a half months in fresh water, during the winter, the mean eosinophil count was significantly lower than that in salt water controls. Approximately 50% of these fish lacked eosinophils in the circulating blood whereas they were never absent in fish kept in salt water. The same trend was observed at the onset of sexual maturation in spring, after a similar period in fresh water, but the decrease was not statistically significant. When breeding fish were kept in fresh water for 16 days, during summer, there was no significant change in the number of eosinophils. Evidently two factors are operative: the length of time in fresh water and the seasonal effect of sexual maturation and regression which either obscures or enhances the primary effect of lowered salinity. Drzewina (1906) subjected two species of marine labrids to gradually decreasing salinity and observed a progressive degranulation and disappearance of eosinophils after 17–20 days when the salinity had been reduced to half that of the sea water used in her experiments.

*Thrombocytes.* As in the case of leucocytes, sexual differences in the thrombocyte count were observed, but these too are difficult to interpret. In wild fish

taken in November the count was significantly higher in females than in males whereas during the breeding season the reverse was true. Under laboratory conditions, no sexual difference was observed during sexual regression, but in the breeding season the females showed significantly higher counts than the males – the opposite of what was found to occur in the natural environment. Seasonal changes are equally conflicting: in wild males the thrombocyte count was lower in winter than in summer whereas in females it was higher. Under laboratory conditions in salt water the males showed no significant seasonal change whereas the females gave significantly higher counts in the breeding season. A similar result was observed in fresh water, but the absolute values were significantly higher for males while females showed no environmental influence. The correct interpretation of these results cannot be elucidated without further investigation.

It should be pointed out that the function of the thrombocytes in the clotting mechanism of fishes is still incompletely understood; Puchkov (1954), for example, has stated that the platelets of fish do not secrete thromboplastin. Katz and Southward (1950) observed, however, that the blood of spent salmon lacks a factor which normally releases thromboplastin from the platelets.

*Hypophysectomy.* In higher vertebrates hypophysectomy is followed by anemia (*e.g.*, Gordon, 1954), and when the pituitaries are removed from *F. heteroclitus* the same phenomenon is evident. A 20–30% decrease in the red cell counts was observed when hypophysectomized fish were compared with sexually regressed intact controls (Fig. 2); the hemoglobin showed a similar trend and the white cell count also declined. Eosinophils were scarce or lacking, depending on the lapse of time after the operation: at three months none could be found in nine fish (Exp. 2). The thrombocytes showed no significant change in hypophysectomized males, but the females showed a significant decrease when compared with regressed controls. Thus, with the possible exception of the thrombocytes, hypophysectomy in this species leads to a chronic state of pancytopenia.

## Changes in the Blood Picture From Stress and Hormonal Injections

Having considered hematological standards for normal fish according to sex and season as well as the effects of hypophysectomy, the experimental work was continued in two directions: (1) a study of the hematological aspects of the stress syndrome in normal fish, and (2) a study of both this and hormonal replacement therapy in hypophysectomized recipients. The discussion of these results is most conveniently organized from the standpoint of cell types.

*Erythrocytes and Hemoglobin.* The minor shock of saline injection had little effect except for an unexplained significant increase in hemoglobin in regressed

females. The more severe shock of immersion in ice water had a delayed action; there was no change after one hour, but after two hours there was a significant decrease in both red cells and hemoglobin. If this response was mediated by way of the pituitary-adrenal axis it might be anticipated that adrenal corticoids or ACTH would have a similar effect. A previous investigation (Slicher, 1951) showed that, in *Tilapia macrocephala*, high doses of ACTH caused a decrease in red cells and hemoglobin. The experimental results from work on *F. heteroclitus* were not decisive; in most instances there was the expected decrease, but cortisol caused an increase in a group of regressed males, and ACTH had no significant effect on breeding females. It may be suspected that these fish were less responsive or that nonpituitary-mediated influences are involved. However, parallel experiments on hypophysectomized fish gave equally conflicting results, hence no clear-cut conclusions can be deduced. In respect to nonpituitary influences, the role of the stressful release of adrenalin must be taken into consideration. In contrast to the rat, Laur (1950) found that adrenalin caused a decrease in the number of circulating erythrocytes in the intact eel and that this effect was reversed after hypophysectomy. No such experiments were made on *F. heteroclitus*, and the opposing action of diverse factors on the abundance of red cells remains an unresolved problem. In the absence of hematocrit determinations, the secondary effects of hemoconcentration cannot be evaluated.

In contrast to the conflicting results of the acute experiments, chronic treatment of hypophysectomized recipients with ACTH revealed a beneficial action on the blood picture. The red cell count regained and even exceeded normal levels for aquarium-kept fish during sexual regression (4,450,000 as compared with 3,940,000) (Fig. 2). That ACTH can alleviate anemia resulting from hypophysectomy was demonstrated in rats by Gemzell and Sjöstrand (1954) and this is now confirmed for fish. Chronic treatment with cortisol, which has a similar beneficial action on the hypophysectomized rat (Gordon and Fruhman, 1955), was not studied in the present investigation.

Other hormones also had a beneficial action. As might be anticipated from the study of normal males during the breeding season, chronic treatment with methyl-testosterone caused a significant rise in the hemoglobin and red cell count of hypophysectomized males; this was correlated with stimulation of the testes and development of nuptial coloration. The role of the thyroid is not clear: although hypothyroidism had little or no effect, chronic administration of TSH relieved the anemia of hypophysectomy. The preparation employed contained sufficient gonadotropin (LH) to stimulate the regressed testes and, in the absence of experiments with thyroxine, these results could be attributed to the release of male hormone. In the frog, Bossak, *et al.* (1948) found that thyroxine had no stimulating action on the peripheral red cell count whereas androgens stimulated erythropoiesis. In general, prolactin had no effect, but in one experiment there was a significant increase in hemoglobin; the combina-

tion of prolactin with ACTH had the same effect as ACTH alone. In a single experiment with intermedin there was a significant decrease in hemoglobin and a lesser, nonsignificant decrease in erythrocytes; prolactin in combination with intermedin reversed these results.

*Lymphocytes.* In laboratory mammals the stress syndrome, mediated through the pituitary-adrenal axis, results in neutrophilia and lymphopenia accompanied by an involution of the lymph glands and thymus (Selye, 1936; Elmadjian and Pincus, 1945). At least one of these responses, the prompt discharge of lymphocytes from the lymphopoietic tissues, has been demonstrated by Rasquin (1951) in a fish, *Astyanax mexicanus*. On the other hand, Slicher (1951, 1958a) found that in *Tilapia macrocephala* high doses of ACTH caused lymphocytosis whereas low doses produced lymphopenia; the degree of lymphocytosis was approximately proportional to the dose. The problem has been further investigated in *F. heteroclitus*. In breeding males and females of this species the mild shock of saline injection, administered under anesthesia, caused a nonsignificant increase in the white cell count two hours later. In contrast normal regressed fish of either sex showed a significant decrease. Furthermore, in such fish the more severe stress of cold shock caused lymphopenia after one hour and lymphocytosis after two hours. Taking these results in conjunction with those on *T. macrocephala*, it may be suggested that regressed fish are less responsive to the mild effect of saline injection in that they show only the initial phase of the stress response (lymphopenia) whereas breeding fish were more sensitive. However, even in hypophysectomized fish, saline injections tended to elicit a mild degree of lymphocytosis, and this could not have been mediated through the pituitary.

Participation of the pituitary-adrenal axis in the lymphocytic response of *F. heteroclitus* to stress is supported by experiments with cortisol and ACTH. Leloup-Hatey (1958) has shown that in agitated carp there is a striking increase of 17-hydroxycorticosteroids in the blood whereas in undisturbed fish the values are very low. In *F. heteroclitus* a single injection of cortisol, the natural circulating steroid of this species (Phillips, 1959), caused lymphopenia in the presumably less responsive regressed males and females; in breeding fish, however, it caused lymphocytosis. The transition from the nonbreeding to the breeding condition was fully supported by experiments on fish taken in the spring, when the nature and degree of the response could be directly correlated with the state of the reproductive organs (Fig. 3). Among those individuals in which the gonadosomatic index was low, cortisol produced lymphopenia, but in more mature specimens it produced lymphocytosis. On the other hand, in hypophysectomized recipients cortisol invariably induced a striking increase in the number of circulating white cells.

It would seem that in normal fish this primary effect of cortisol on the lymphopoietic organs may be masked by interactions which evidently involve

the pituitary and which are further complicated by the state of maturation of the gonads. At the relatively high doses employed, ACTH invariably caused lymphocytosis, but it may be inferred from previous work on *T. macrocephala* and from the early phase of cold shock in *F. heteroclitus* that low doses would result in lymphopenia. Evidently there is an interaction between two competing factors, one favoring lymphopenia, the other lymphocytosis. The present investigation establishes only the probable existence of these two opposing factors and indicates that the function of the pituitary is involved. The mammalian literature provided no clue to an interpretation of these findings since it is well known that adrenal corticoids cause lymphopenia even after hypophysectomy (Fruhman and Gordon, 1956; Gerstnor and Gordon, 1958). It must be remembered that in both *F. heteroclitus* and *T. macrocephala* there are no neutrophilic granulocytes, hence no comparison can be made with the neutrophilia of the stress reaction in mammals.

As already pointed out, chronic treatment of hypophysectomized recipients with ACTH resulted in a marked increase in the white cell count. Neither thyrotropin (containing LH) nor methyl-testosterone relieved the lymphopenia of hypophysectomy, even after one month treatment. In one experiment, prolonged administration of purified prolactin had no significant effect, but in combination with ACTH there was the expected lymphocytosis; in a second experiment, under the same conditions, another preparation caused a significant increase in the white cell count. Intermedin also produced a significant increase, as did the combination of intermedin and prolactin. Hypothyroidism caused a significant decrease in the white cell count of intact fish in salt water and a similar but lesser effect in those maintained in fresh water.

*Eosinophils.* It is well known that in mammals the stress syndrome results in eosinopenia, but little is known regarding the response of poikilotherms. Nardone and St. John (1956) found in the turtle, *Pseudemys elegans*, that cold caused a decrease in the number of circulating eosinophils and that cortisone had the opposite effect. The blood was sampled $4^1/_2$ hours after treatment. In normal regressed *F. heteroclitus* cold shock resulted in a decrease in the percentage of eosinophils one hour after treatment and an increase after two hours. These changes followed the same pattern as the lymphocytes. The milder shock of saline injection also elicited eosinophilia two hours subsequently, except for a group of regressed females which showed no significant change. High doses of cortisol caused eosinopenia in normal regressed fish of either sex, eosinophilia in breeding males; no breeding females were tested. The seasonal reversal of the response is similar to that of the lymphocytes (discussed in the preceding section) and in spring fish the reaction may be similarly correlated with the gonadosomatic index. In regressed winter males, ACTH caused eosinophilia, but during the breeding season there was no change in the percentage; however, taking into account the enormous increase in the

total number of white cells, the absolute values for the eosinophils were also elevated.

The pancytopenia of hypophysectomy in *F. heteroclitus* is accompanied by a progressive decrease in the percentage of circulating eosinophils; after two to three months these cells are rarely if ever seen in the blood smears. In such fish, hypophysectomized 2–6 months, injections of saline, cortisol or ACTH resulted in a reappearance of circulating eosinophils two hours later (Exps. 4, 7), but the response from ACTH was less than that from saline injections (controls). The evidence indicates, furthermore, that the reappearance of circulating eosinophils is of a transitory nature. In two chronic experiments (Exps. 9, 20) these cells were not observed in specimens autopsied 24 hours after the last saline injection. Apparently hypophysectomy does not obliterate eosinophils from the tissue reservoirs. Some factor must exist in the hypophysectomized animals which prevents, or fails to elicit, the release of these cells into the blood stream, and this barrier is transitorily reduced after the injection of saline. Clearly the stimulus does not operate through the pituitary, and release of adrenalin under stress may be suspected. In the turtle, Nardone and St. John (1956) found that injection of adrenalin caused eosinophilia. In mammals there has been a controversy regarding the fate of the eosinophils which disappear under stress. However, in the rat, Wegelius and Teir (1958) have given convincing evidence of tissue storage rather than lysis of the eosinophils. The reappearance of eosinophils in the chronic experiments with ACTH and prolactin, and to a limited extent even with TSH or methyl-testosterone, is possibly subject to a different interpretation: autopsies were made 24 hours after the last injection, and the saline controls lacked eosinophils. Hormonal influences, under these circumstances, therefore maintain a chronic lowering of the barrier to eosinophil release, correlated with an over-all improvement in the blood picture. Chronic administration of intermedin alone failed to restore the circulating eosinophils and, in combination with prolactin, this hormone apparently had an inhibitory effect: only two fish out of ten showed the presence of these cells in small numbers in the blood stream (1% and 2% respectively).

*Thrombocytes.* The conflicting experimental data provide no grounds for constructive discussion regarding the regulation of the abundance of thrombocytes.

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Эндокринологическое и гематологическое изследование рыбы *Fundulus heteroclitus* (Linn.).

## Краткий обзор

Кровь *Fundulus heteroclitus* (Linn.) содержит следующие типы клеток: эритроциты, лимфоциты, эозинофилы и тромбоциты. Базофилы и нейтрофилы отсутствуют. В течении сезона размножения количество гемоглобина и число эритроцитов у самцов выше чем у самок. Во время половой регрессии число эритроцитов у самцов понижается, а у самок возрастает до одинаковаго уровня. У рыб содержавшихся в лаборатории при 20° С. количество гемоглобина и число эритроцитов были выще чем у рыб живших в их естественной окружающей среде. У рыб содержавшихся несколько месяцев в пресной воде количество гемоглобина и число эритроцитов были выше чем у рыб содержавшихся в соленой воде и у них наступал слабый лимфоцитоз с заметным понижением числа циркулирующих зритроцитов. Гипотиреоз причиненный радиоактивным иодом или вовсе не изменял, или только незначительно изменял картину крови. Панцитопения наступавшая после хирургическаго удаления гипофиза облегчалась по крайней мере у самцов хроническим применением АКТГ, метилтестостерона или тиреотропнаго гормона содержащаго лютеинизирующий гормон. Пролактин был менее эффектен, а интермедин не оказывал никакого влияния. Изменения в количестве гемоглобина и в числе зретроцитов вызванные раствором соли, холодовым шоком, кортизолом и АКТГ давали противоречивые результаты. Холодовый шок давал характерную картину по сравнению с обилием лейкоцитов в крови нормальных рыб, а именно появление лимфопении через один час и лимфоцитоза через два часа. Резузьтаты других способов воздействия могут быть обьяснены на основании этой двухфазной реакции. Легкий шок (иньекция раствора соли) причинял появление первой фазы. Большия дозы АКТГ вызывали лимфоцитоз. Кортизол имел тот или другой эффект в зависимости от половой зрелости рыбы: лимфопения во время половой регрессии, лимфоцитоз при наступлении половой зрелости. После хирургического удаления гипофиза появление только второй фазы могло быть вызвано. Изменения в числе эозинофилов обычно наступали вслед за реагированием лимфоцитов. Спустя более двух месяцев после удаления гипофиза эозинофилы исчезали из тока крови. Иньекция раствора соли, АНТГ или кортизола вызывала их кратковременное возвращение. Их окончательное возстановлние совпадало с улучшением гематологической картины крови в результате хроническаго применения АНТГ или пролактина. Интермедин подавлял воздействие этих гормонов.