UNITED STATES DEPARTMENT OF THE INTERIOR, Fred A. Seaton, *Secretary*
FISH AND WILDLIFE SERVICE, Arnie J. Suomela, *Commissioner*

ACCELERATED DEVELOPMENT OF TESTIS AFTER UNILATERAL GONADECTOMY, WITH OBSERVATIONS ON NORMAL TESTIS OF RAINBOW TROUT

By O. H. Robertson, M. D.

FISHERY BULLETIN 127
From Fishery Bulletin of the Fish and Wildlife Service
VOLUME 58

UNITED STATES GOVERNMENT PRINTING OFFICE • WASHINGTON • 1958

For sale by the Superintendent of Documents, U. S. Government Printing Office
Washington 25, D. C. Price 25 cents

ABSTRACT

An experimental study was made of the growth and development of the testes in rainbow trout employing unilateral gonadectomy to stimulate spermatogenesis in the infantile gonad. It was found that the remaining testis of trout more than a year old increased rapidly in weight and within a period of 6 weeks had grown to 3 or 4 times the size of a single testis in the control fish. Histological studies showed that the cellular changes occurring in these experimentally stimulated testes were identical with those found in the testes of trout maturing normally. These are described in detail. Failure of young trout to exhibit growth of the testis remaining after unilateral gonadectomy was found to be due not to lack of stimulability of the juvenile testis but most probably to the nonreactivity of the pituitary at this age. Good nutrition was observed to favor, while starvation suppressed, testis growth.

CONTENTS

ACCELERATED DEVELOPMENT OF TESTIS AFTER UNILATERAL GONADECTOMY, WITH OBSERVATIONS OF THE NORMAL TESTES OF RAINBOW TROUT

By O. H. ROBERTSON, M. D., *Department of Biological Sciences, Stanford University, Stanford, Calif.*

While much has been learned about the process of fertilization of the egg, formation of the embryo and the hatching of fishes, relatively little study has been given to the development of the fish's sex glands and the factors which influence their growth and maturation. This is especially true of salmon and trout and is in marked contrast to the wealth of information available on the reproductive cycles in mammals, birds, and amphibians. Precise knowledge concerning the sequence of developmental stages of the gonads in the salmonid fishes is needed, not only for a clearer understanding of the mode of formation of sperm and ova, but is essential for the valid interpretation of experimental procedures directed toward influencing the rate of sexual maturation.

Fishery biologists in various parts of the world have been experimenting on speeding up development of sex products with the purpose of increasing the production of certain fishes. The method usually employed has been injections of pituitary glands. Results have varied with the species of fish tested and the criteria used for judging the effect. Such procedures have been only moderately promising in salmon and trout thus far, but would seem to be worth pursuing further in view of the urgent need in certain regions of this country to maintain the populations of Pacific salmons and sea-run trout. That more knowledge of the normally developing salmonid is necessary before studies of this nature can be successfully undertaken was discovered by the author and A. P. Rinfret when initiating an investigation of the effect of pituitary substances administered to juvenile rainbow trout, *Salmo gairdnerii* (1957).

Our observations soon showed that an appreciable percentage of young male trout exhibited spontaneous enlargement of the testes at an early age. To be certain of the gonad-stimulating action of any procedure in individual trout, it was obviously necessary to determine the size of their testes beforehand. Unilateral gonadectomy undertaken for this purpose revealed the unexpected finding of well marked ensuing increase in the size of the remaining testis in the control (uninjected) fish. This stimulation of testicular growth presented a further complication in estimating the effect of pituitary hormones on the trout's gonads but at the same time it provided another means of studying the process of gonadal development.

The present investigation was undertaken with the purpose of learning more about the natural course of sexual maturation in male rainbow trout and determining some of the factors which influence the inception and rate of spermatogenesis.

This work was aided by grants from the American Academy of Arts and Sciences and the U. S. Fish and Wildlife Service.

Especial appreciation for his invaluable aid is due my good friend and neighbor, John McCarthy, who installed the water supply to the laboratory, acts as engineer, and takes care of the experimental fish during my absences. It is a pleasure to record my indebtedness to George Dufour of Santa Cruz, Calif., for his generous cooperation in supplying trout of desired size and age throughout the past 3 years. The fact that his trout were free from disease and in excellent condition greatly facilitated the present investigation. He also provided ground liver. To Dr. Clifford Sweet of Oakland, Calif., I want to express my appreciation for suggesting the type of suture employed in the operative wound closure and for instructing me in its use.

NOTE.—Approved for publication February 18, 1957. Fishery Bulletin 127.

Thanks are due to the Upjohn Company of Kalamazoo, Mich., for the penicillin which was provided through the kindness of Dr. Jaret H. Ford. Finally, I wish to acknowledge my obligation to John Daniels of the Department of Medicine, University of Chicago, for the large number of excellent histological preparations employed in this study.

METHODS OF STUDY

Experimental Trout

The trout were obtained from the private hatchery of George Dufour, located near Camp Evers in the Santa Cruz mountains, Calif. They were hatched from eggs obtained from the brood stock maintained at Logan, Utah.

Environmental Conditions

The experiments were conducted in the author's laboratory in the Santa Cruz mountains, where an adequate supply of spring water was fed into glass-walled aquaria ranging from 10 to 18 gallons capacity. Partitions separating individual trout were found to be necessary to prevent trout from injuring each other. These consisted of ⅝-inch mesh galvanized screen held in place on the bottom by steel bars—all coated with aluminum paint. The small space allotted to each fish did not appear to be detrimental to their well being since the trout grew at the rate of 0.2 to 0.3 inch per week and remained free from fungus infection. The water temperatures ranged from 48° to 50° F. in winter to 58° to 60° F. in summer with infrequent brief rises to 65° F. A flow of water was maintained which gave a complete exchange every 30 minutes. In late summer and fall this was reduced somewhat and aeration of the water was instituted with small air pumps. Uneaten food and excreta were removed daily by means of a suction tube emptying into the outflow trough under the aquaria. A complete change of water was made following a thorough weekly cleaning.

Food

The trout were fed 6 days of the week on dried pellets provided by the U. S. Fish and Wildlife Service through the kindness of Dr. Arthur M. Phillips, Jr., Cortland, N. Y. (1953). On the seventh day ground liver was fed.

Length and Weight Measurement

The trout were measured to the nearest 0.1 inch, total length,[1] and weighed to the nearest 0.5 gram, once a week under anesthesia produced by immersion in water containing a 1:20,000 dilution of tricaine methanesulphonate, M. S. 222. A sufficient degree of anesthesia occurs usually in 2 to 3 minutes. Recovery after returning to the aquarium takes place equally rapidly. No harmful effects from this anesthetic have been observed even after daily use for a number of weeks.

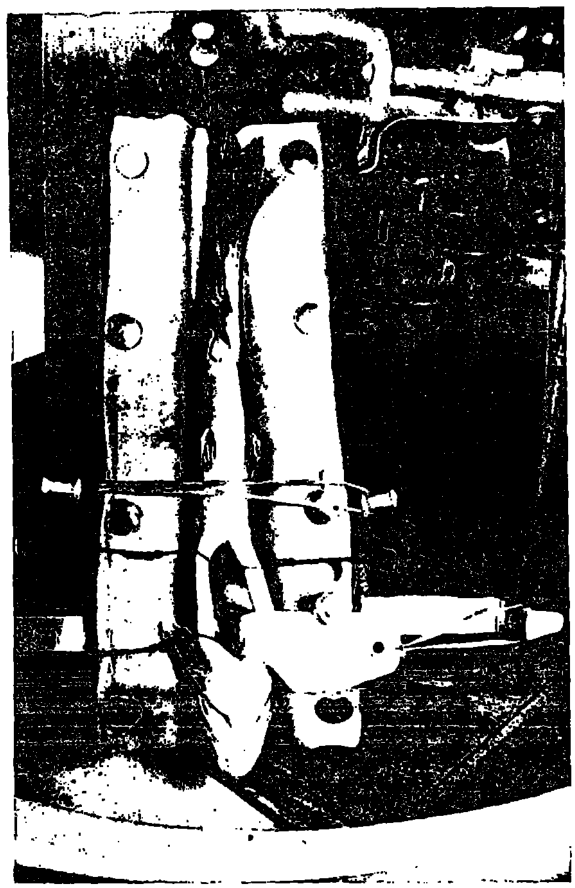

FIGURE 1.—Trout on operating board with head submersed in anesthetic solution to a level just above gills. Retractor in place.

[1] Fork length measurement was also made but total length in trout of this age and size was more satisfactory.

FIGURE 2.—Retractors for use on right and left sides.

Operative Technique

The trout having been starved 48 hours was anesthetized in a 1:20,000 dilution of M. S. 222 then placed on the operating board that dipped into a solution of 1:25,000 solution of the same anesthetic. To hold the fish on a tilting board (fig. 1) a No. 10 fishhook, with barb removed, was inserted through the skin near the anal fin and by means of an attached rubber band was pegged to the board in the desired position. The sides of the trough were placed sufficiently far apart so as not to interfere with the gills which should be completely submerged. In order to operate in the peritoneal cavity without assistance, somewhat special arrangements had to be provided: these consisted principally in the construction of retractors made of stainless steel (fig. 2), which could be held in place by means of an attached rubber band stretched over a stainless steel arm fastened to the operating board as shown in figure 1. An aluminum push pin helps hold the tractor in place. A single rubber band over the pelvic region of the trout suffices to hold the fish on the board. The instruments were sterilized in 70 percent alcohol.

Incision circa 3 cm. long in a 7- to 8-inch fish was made in the mid-line which resulted in little or no bleeding, if terminated at a point just posterior to the insertion of the pectoral fins. Four barbless fishhooks attached to small lead sinkers served to hold the body walls apart. A focusing head mirror was used to illuminate the abdominal cavity. The shaped retractor made it possible to hold the liver away from the cephalic attachment of the testis. A clearly visualized field in this area is essential since complete excision is necessary to prevent regeneration. The only important precaution to be observed at this stage is to avoid injury of the swim bladder against which the testis lies. The greater portion of the gland can be removed through this relatively small opening by pulling the gonad as far forward as possible then slipping the scissors into the abdominal cavity along its stretched caudal end. No regeneration of the remaining portion, principally spermatic duct, has been observed within the experimental period. Before closing the wound a capsule containing 5 to 10 mg. of penicillin powder was emptied into the peritoneal cavity.

The incision was sewed up with No. 5–0 chromic gut and a six-eighths of an inch or seven-eighths of

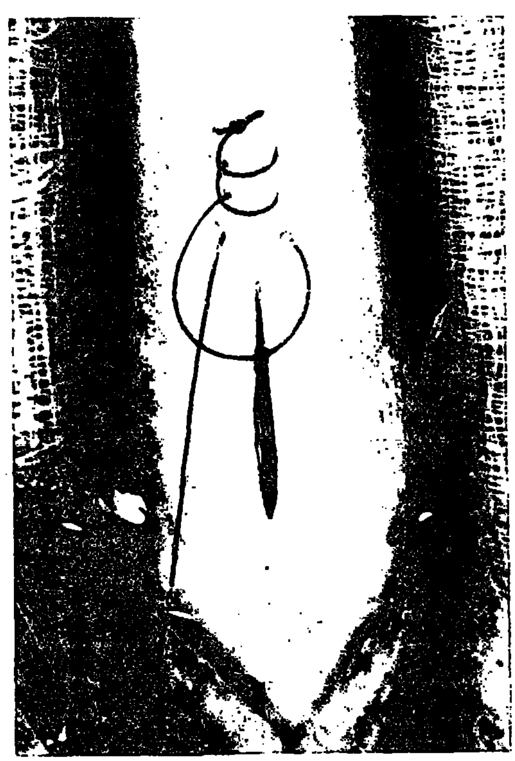

FIGURE 3.—Procedure of beginning lock stitch.

an inch straight cleft palate needle. The type of suture used known as lock stitch produced a close and even approximation of the two edges. After trying the first stitch the next was made on the right side of the incision (operator's right) passing the needle completely through the body wall about one-sixteenth of an inch from the edge, then up through the wall of the opposite side. The needle was then passed inside the diagonal loop of the preceding stitch before making the next one. Figure 3 illustrates this part of the technique. Eleven to 13 stitches were used to close a 3-centimeter incision, each one pulled snug before the next was made. It was found that stitches thus placed resulted in more rapid and even healing than when spaced further apart. The final tie was made with the short end of the suture and the needle or double end as in figure 4.

Chromic gut will begin to absorb in 2 to 3 weeks and has usually disappeared by the end of the fourth week. No post-operative care was found necessary other than withholding food for 48 hours. The mortality attributable to the operative procedure was no more than 1 or 2 percent.

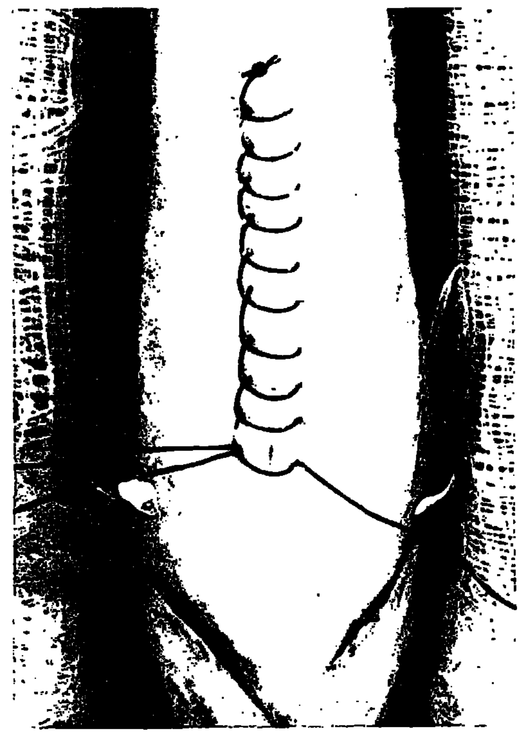

FIGURE 4.—Procedure for finishing lock stitch. The two ends are ready to tie.

EXPERIMENTAL STUDY OF DEVELOPMENT OF TESTES

Since no quantitative data on the growth and development of the testes in juvenile rainbow trout are to be found in the literature, pertinent observations were made on a large number of normal trout of different lengths and varying ages. Then a study of unilateral gonadectomy was undertaken with the objective of describing the phenomenon of accelerated growth of the remaining testis and relating the experimentally induced spermatogenesis to the normal development of the testes in rainbow trout.

Relation of Testis Weight to Length, Weight, and Age of Trout

In relation to testes weight it was found that the length of the fish was a much more informative index than was its weight. Individual trout, especially those kept in large rearing ponds, may fluctuate in weight quite rapidly, depending on the amount of food they obtain. Examination of a consecutive series of 253 trout 7 to 16 months of age, freshly taken from the hatchery rearing ponds, revealed 240 to be sexually immature and 13 with marked testicular development. The weights of the immature testes were plotted as shown in figure 5. Since this investigation dealt largely with the results of unilateral gonadectomy, one-half the weight of each pair of testes or that of a single gonad was used in making the graph. Several features of this graph will be noted at once. First the great majority of the points are clustered closely along a median line (drawn by inspection). Second, there is a gradual increase in testis weight with increasing length of the trout until it reaches about 8 inches when the testis weight curve rises more sharply with the trout's further growth. Trout greater than 10 inches in length were not included because of space considerations in the aquaria.

The importance of length versus age immediately comes to mind in this connection. Is this curve a valid one for trout of the same size but of widely differing ages? To answer this question, the weights of immature testes in trout of two length categories 6.5 to 7.4 inches and 7.5 to 8.4

inches were plotted against age as shown in figs. 6 and 7. It is seen that between the ages of 7 and 16 months the size of the testis is essentially the same in fish of similar length, i. e., growth of the infantile testes parellels growth of the other components of the trout until the process of gonadal maturation begins to occur more generally after 16 months of age (fig. 8).

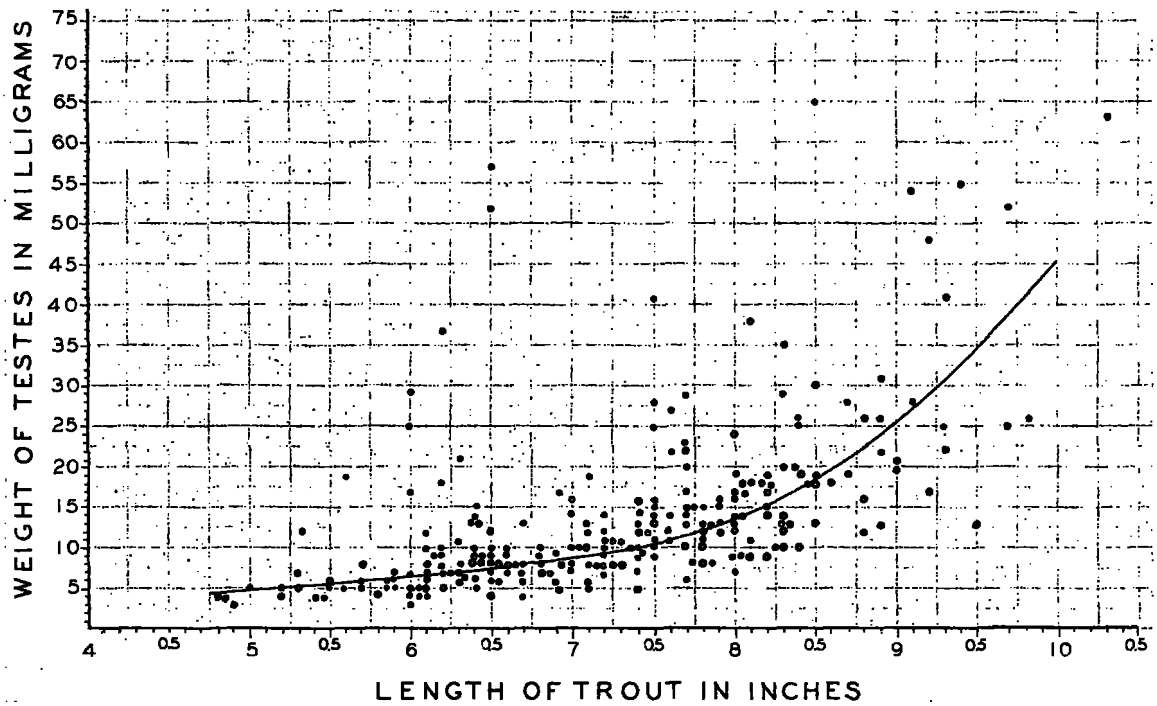

FIGURE 5.—Weight of immature testes in 240 rainbow trout ranging from 4¾ to 10 inches in length and 7 to 16 months old.

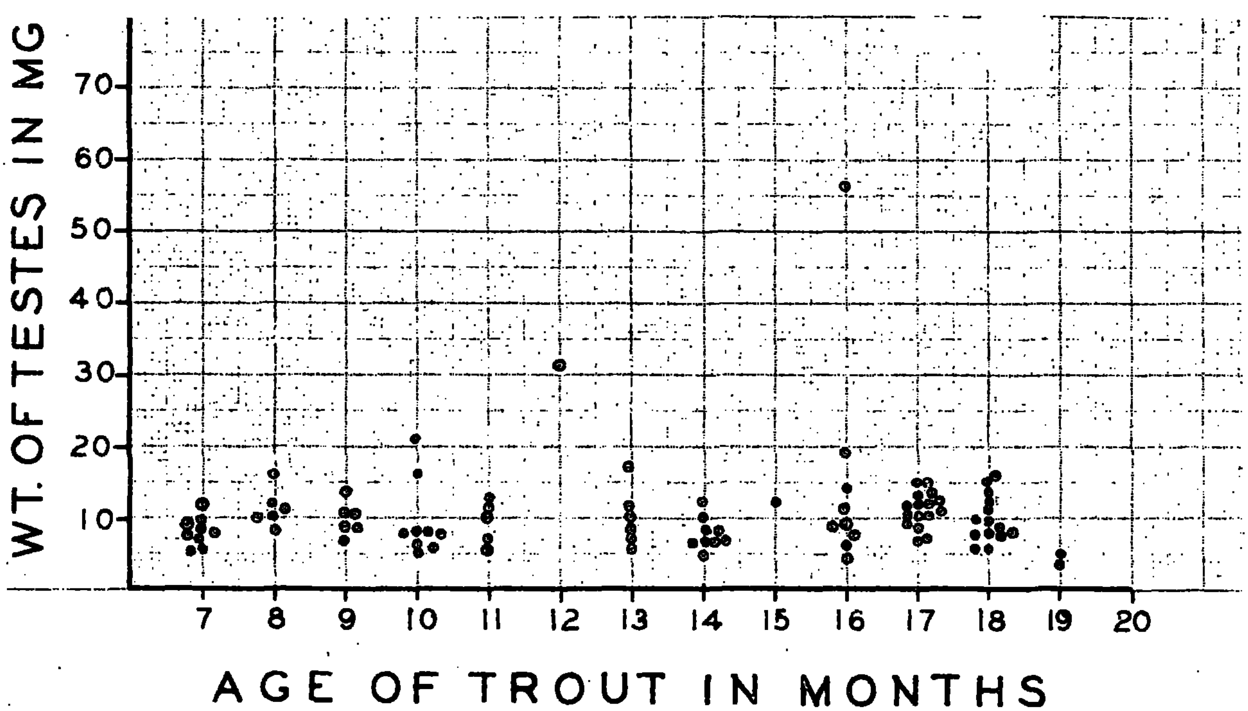

FIGURE 6.—Age of fish versus weight of testes in trout 6.5–7.4 inches in length.

435916 O—58——2

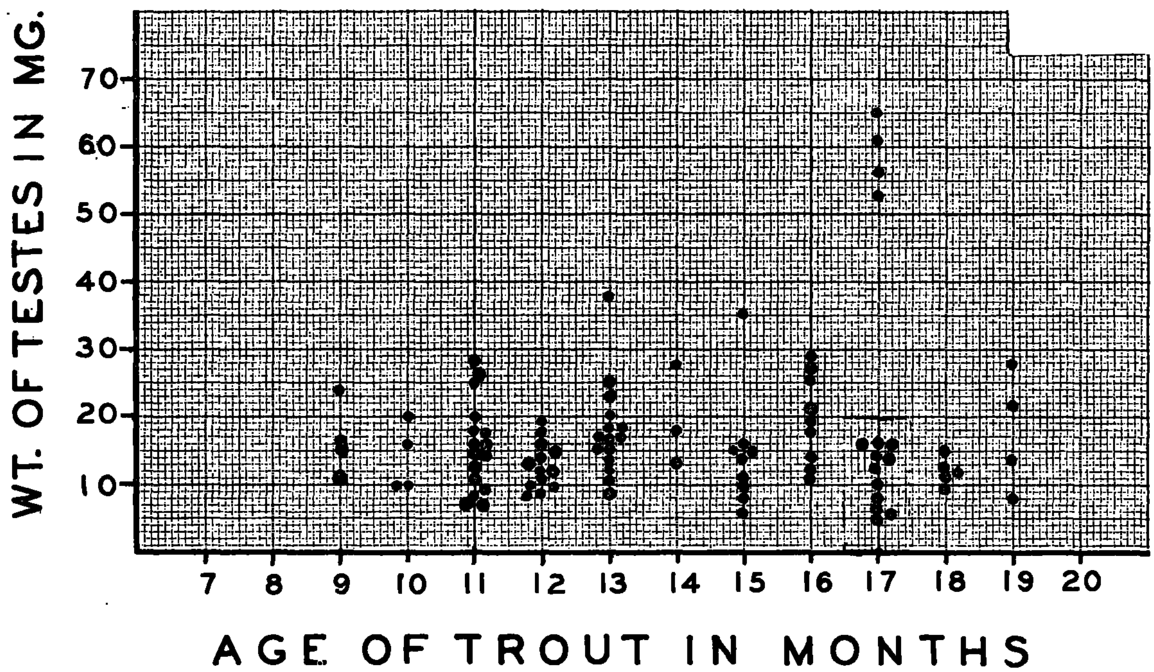

FIGURE 7.—Age of fish versus weight of testes in trout 7.5–8.4 inches in length.

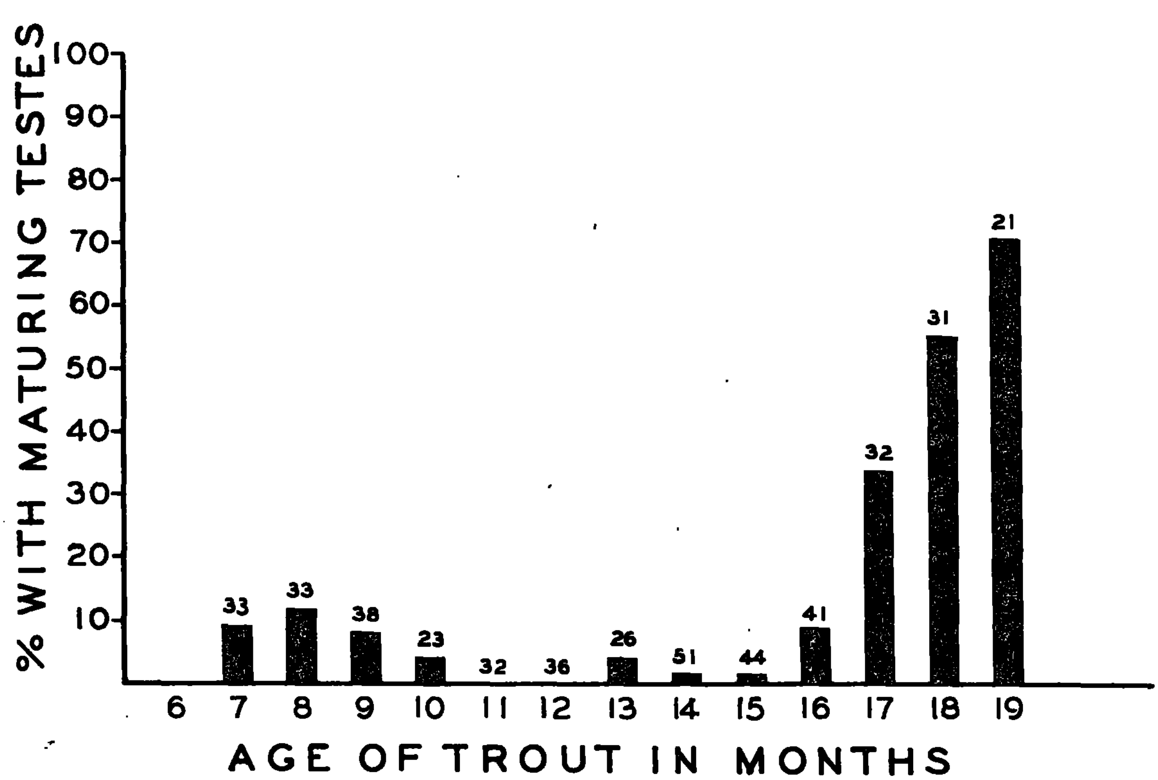

FIGURE 8.—The percentage of young rainbow trout exhibiting beginning development at successive months of age.

Occurrence of Precocious Sexual Maturity

The observed not infrequent occurrence of very large and sometimes fully mature testes in trout as young as 6 or 7 months of age indicated the importance of determining how frequently this event took place. Data on the percentage of mature trout at different ages in months based on some 350 fish hatched in 1952 and 1953 and collected over a period of 2½ years, are graphed in figure 8. The designation of "trout with developing testis" includes those fish in which single gonad weight exceeded the highest point on the curve in figure 5, namely 65 milligrams. Actually all these trout exhibited testes weights many times that of the normal growth curve. There was no essential difference between the two year classes of trout so they were combined without distinction on the graph.

It is of interest that early sexual maturity occurred more frequently in trout from 7 to 9 months of age than at later ages up to 16 months.[2] The absence of any fish showing gonadal development in the 11- to 12-month age group was observed each year. Beginning with 17 months an increasing percentage of trout exhibited testicular growth. However, a certain portion of male trout were found to have infantile gonads up to and beyond the age of two years.

Preponderance of Left Testis Weight

In order to be able to estimate the weight of the testis remaining after unilateral gonadectomy it was necessary to determine the average weight of the right and left testis. Weights of immature testes in 147 trout from 6 to 16 months of age revealed that the average weight of the left testis was approximately 15 percent (14.8) greater than that of the right. When testes weights of 35 of similar age but from another stock were added to the above group the preponderance of L/R rose to 16 percent.

The average weights of testes in varying stages of development up to full maturation (but not oozing milt) in 73 rainbow trout of ages from 6 months to 12 months showed a weight preponderance of L/R of 16.8 percent. The combined average left and right testis weights in all 255 trout gave a left preponderance of 16.3 percent.

Results of Unilateral Gonadectomy

The first series of gonadectomies were performed in January 1953 on trout 11 to 12 months old. Autopsy after 43 to 48 days revealed a striking increase in the size of the remaining testes in 3 of the 6 experimental fish and a moderate though definite increase in the others. Changes in weight from 4 mg. estimated at time of operation to 108 mg. found at autopsy, and from 15 mg. to 186 mg., occurred. For the 3 trout exhibiting the largest remaining testis, the gains in weight ranged from 1,100 to 2,450 percent. For the group of 6 fish, the percentage gain was 1,000. Controls consisting of laparotomy and inspection of testes were not run coincident with this series. However, 3 such fish operated on 6 weeks previously showed 46 days later testes of approximately the size expected from the testes growth curve (fig. 5).

A second series of unilateral gonadectomies carried out in July and August of the same year on trout of the same year class as the first series but now 17 to 18 months of age, resulted in tremendous growth of the remaining testes: e. g., from an estimated 6 mg. at the time of operation to 3,065 mg. found at autopsy 7 weeks later, more than 500-fold increase in weight, table 1. However, simultaneous controls, operated and inspected only, showed equally marked growth of testes (table 2). Whether the difference between the results of the two experiments (winter and summer) was due to the greater age of the trout in the second series and hence the likelihood of imminent spontaneous testicular maturation as indicated in figure 8, or to some other factor or factors, was not clear. The observed absence of gonadotrophin production by the infantile pituitary in some mammalian species under a certain age suggested that the age of the trout might well have an important influence on its response to unilateral gonadectomy, assuming that the underlying mechanisms is one of pituitary stimulation.

---

[2] Examination of thirty nine 6½- to 7-month-old trout of the 1955 hatch showed 15 percent of them to be in an advanced stage of testicular maturation. These trout were from a different source than those represented in figure 8 so were not included.

TABLE 1.—*Change in weight of remaining testes following unilateral gonadectomy in trout 18 months of age*

[Period of observation 7 weeks]

Trout No.	Length changes	Weight of excised testis	Estimated weight of remaining testis	Remaining testis at autopsy: Weight	Remaining testis at autopsy: Net gain [1] in weight	Gain in length of trout
	Inches	*Mg.*	*Mg.*	*Mg.*	*Percent*	*Percent*
137	5.2–6.3	R 3	L 3	[2] R 274 L 1,351	44,933	21
138	5.5–6.1	L 4	R 4	R 10	225	11
139	4.7–6.1	L 7	R 6	R 740	12,217	30
141	7.4–8.7	R 12	L 14	L 1,586	11,143	18
151	6.1–7.4	R 5	L 6	L 3,065	50,933	21
153	6.1–6.9	R 4	L 4	L 14	200	13
154	6.1–7.2	L 15	R 13	R 2,305	17,608	18
155	6.0–7.1	L 33	R 29	R 2,180	7,410	18

[1] The net percentage gain in weight of testes is calculated on the basis of the actual gain (weight at autopsy less estimated weight at operation) minus the expected gain in weight accompanying growth in length of trout as shown in fig. 5.
[2] Regenerated from tiny fragment which escaped excision.

TABLE 2.—*Change in weight of testes following laparotomy and inspection of testes in trout 19 months of age*

[Period of observation 6 weeks]

Trout No.	Length changes	Appearance of testis at operation	Estimated weight of single testis	Testes at autopsy: Weight	Testes at autopsy: Net gain in weight of single testis [1]	Gain in length of trout
	Inches		*Mg.*	*Mg.*	*Percent*	*Percent*
156	7.3–8.4	Thread-like.	9	R–2950 L–4670	42,333	15
157	6.5–7.0	...do...	7	R–1730 L–1880	18,640	8
158	7–3–8.9	...do...	9	R–2085 L–2800	26,960	22
159	6.9–8.0	...do...	8	R–2680 L–3840	40,675	16
160	6.9–7.7	...do...	8	R–2460 L–2460	30,710	12
161	7.6–9.1	...do...	11	R–2590 L–4130	30,400	20
164	7.7–8.2	...do...	12	R–2000 L–2030	16,765	19
165	6.8–8.7	...do...	8	R–4310 L–5410	60,610	27

[1] Taken as ½ weight of the 2 testes.

In the next experimental series, observations were begun when the trout were 7 months of age and continued first at two monthly then at monthly intervals until the age of 17 months. The summarized results of 7 experiments comprising 78 trout, about equally divided between test and controls, are shown in table 3. Detailed data of one group, trout 16 months of age, are displayed in tables 4 and 5. Before the age of 13 months there appeared to be no significant difference between the test and the control trout; both showed definite though very moderate increase in testicular weight as compared with normal trout of the same length.[3] At 11 months the combined weights of the 2 testes of the controls were approximately twice that of the single testes of the unilaterally gonadectomized fish. At 13 months, however, the remaining gonad of the test fish was found to be about the same weight as the two testes of the control. This result was repeated at 14 and 15 months but at 16 months the remaining testes of the gonadectomized trout averaged twice the combined weight of the 2 glands of the controls. Figures 9 and 10 are representative of the appearance of the testes in the experimental and control trout at 16 months of age. In figure 9 a small regenerated remaining fragment of the excised right testis is shown between it and the enlarged left testis found at autopsy.

TABLE 3.—*Effect of unilateral gonadectomy at different ages on the growth of the remaining testis*

Age of trout at autopsy (months)	Unilateral gonadectomy: Number in each group	Remaining testis at autopsy: Average weight	Remaining testis at autopsy: Average net gain weight	Controls: Number in each group	Testes at autopsy: Average combined weight both testes	Testes at autopsy: Estimated net gain weight single testis
		Mg.	*Percent*		*Mg.*	*Percent*
9	4	25	286	6	34	136
11	4	36	262	5	78	288
13	6	78	407	5	74	196
14	6	73	499	7	80	171
15	6	63	620	6	94	346
16	5	206	1,373	7	118	545
17	6	442	7,975	6	412	1,995

TABLE 4.—*Change in weight of remaining testis following unilateral gonadectomy in trout 16 months of age* [1]

[Period of observation 6 weeks]

Trout No.	Length changes	Weight of excised testis	Estimated weight of remaining testis	Remaining testis at autopsy: Weight	Gain in weight	Net gain in weight	Gain in length of trout
	Inches	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Percent*	*Percent*
328	6.3–7.3	L 22	R 19	R 292	273	1,422	16
329	6.2–6.9	L 7	R 6	R 12	6	66	11
332	6.2–7.4	R 17	L 20	L 335	315	1,560	19
333	6.0–7.1	R 5	L 6	L 104	98	1,600	18
338	7.2–8.4	R 10	L 12	L 286	274	2,217	17

[1] Trout 15 months old when operated on.

NOTE.—Average gain in length of trout, 18 percent. Average gain in weight, 87 percent.

[3] Errors arising from the estimated weight of the remaining testes in the unilaterally gonadectomized trout (see footnote to table 1) are probably not significantly different from similar errors in estimating by the same means the weight of the testes in the inspected controls. There seemed to be no better way of devising a control on the effect of the removal of one gonad.

TABLE 5.—*Change in weight of testes following laparotomy and inspection of testes in control trout at 16 months*[1] *of age*

[Period of observation 6 weeks]

Trout No.	Length changes inches	Appearance of testis at operation	Estimated weight of single testis	Testes at autopsy: Weight	Single testis: Gain in weight	Single testis: Net gain in weight	Gain in length of trout
	Inches		*Mg.*	*Mg.*	*Mg.*	*Percent*	*Percent*
330	6.6–7.6	Stringlike	8	R 67, L 76 } 71	63	750	15
331	6.0–7.2	...do	7	R 10, L 9 } 10	0	0	20
334	6.4–7.3	...do	7	R 42, L 45 } 43	36	471	14
336	6.2–7.1	...do	7	R 112, L 105 } 109	102	1,430	15
340	6.7–8.0	...do	8	R 17, L 23 } 20	12	75	19
341	7.3–9.0	...do	10	R 59, L 68 } 63	53	380	23
342	7.2–8.9	...do	10	R 77, L 113 } 95	85	710	24

[1] Trout 15 months old when operated on.

NOTE.—Average gain in length of trout, 19 percent. Average gain in weight, 107 percent.

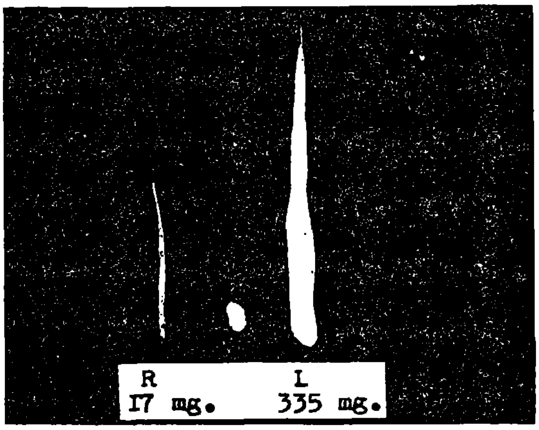

FIGURE 9.—Testes of rainbow trout No. 332, table 4. Right testis (R) excised. Left testis and regenerated fragment of right testis, weight 19 mg., found at autopsy 6 weeks later. Trout 16 months old when killed.

The percentage gain in weight gives a more accurate comparison of testicular growth on the test and control groups since this figure takes the size of the fish into account. Thus at 16 months of age the weight gain of the test-trout gonad is between 2 and 3 times that of a single testis in the control group and not 4 times as would be indicated by comparing weights alone (table 3). At 17 months of age the percentage gain in weight of the testes in the gonadectomized trout is about 4 times that of the controls but at this age a considerable percentage of the trout were manifesting spontaneous testicular maturation.

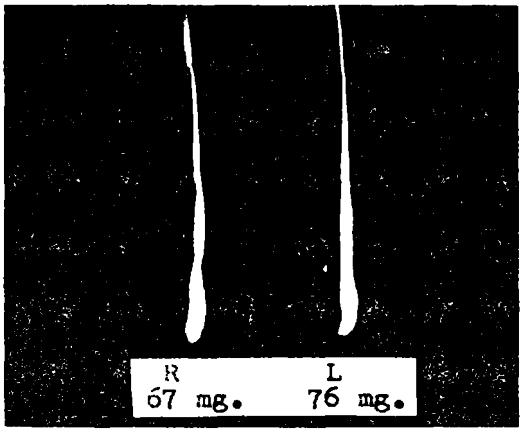

FIGURE 10.—Testis of control rainbow trout No. 330, table 5. Killed 6 weeks following simple laparotomy and inspection of testes which were seen to be infantile. Age at death 16 months.

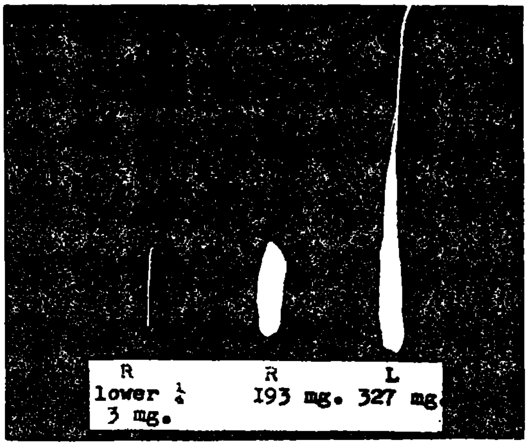

FIGURE 11.—Testes of rainbow trout No. 343 (table 6) six weeks following partial unilateral gonadectomy; caudal, one-fourth of right testis excised. Age at death 16 months.

Partial Unilateral Gonadectomy

In order to determine whether the degree of growth stimulation of the remaining testis depended on the quantity of testicular substance removed, varying portions of one testis were exercised from a series of 16 months old trout. The results of this experiment (table 6) show that the excision of as little as one-fourth (estimated) of one testis was followed by marked increase in weight of the other intact testis, as well as growth of the remaining part of the partially excised one (fig. 11). No essential difference in effect was observed from removal of one-fourth or three-fourths of a testis. The average total testicular mass of the remaining intact testis in these 5 trout was about the same as observed in a group of the same age in which a whole testis was excised (table 4).

TABLE 6.—*Effect of partial unilateral gonadectomy on the growth of the remaining testes in trout at 16 months of age*

[Period of observation 6 weeks]

Trout No.	Length changes	Excised tests		Estimated weight of remaining testis	Remaining testis at autopsy	
		Amount estimated	Weight		Weight	Net gain in weight of intact testis
	Inches		*Mg.*	*Mg.*	*Mg.*	*Percent*
337	6.7–7.5	Caudal ¼ of left	3	8	R 33 L 17	288
343	7.1–7.7	Caudal ¼ of right	3	9	L 327 R 193	3.500
335	6.5 7.6	Caudal ½ of left	6	7	R 168 L 52	2.200
344	7.0–8.7	Caudal ½ of right	5	9	L 164 R 53	1.590
339	6.6–7.8	Caudal ¾ of left	5	7	R 111 L 23	1.414

NOTE.—Average total testicular mass=228 mg.: average percentage net gain in weight of intact remaining testis=1.738.

Growth of Testes in the Control Trout

The gain in weight of the testis in the control trout observed throughout the series of experiments summarized in table 3, suggests that laparotomy itself may have a stimulating action on testis growth. This weight gain was fairly constant, ranging from 136 percent to 288 percent until the age of 15 months when it began to increase and by 17 months, the testis, during the 6 weeks of observation increased its weight twentyfold. There are, however, factors other than operation which undoubtedly contribute to accelerated gonadal growth in these fish. Trout taken from the hatchery rearing ponds containing large populations of fish and placed in individual compartments in the laboratory aquaria begin to grow at a greatly increased rate. The possibility that such rather sudden change in metabolism might stimulate beginning development of the male gonad was tested by placing a group of 13–16-month-old trout in the aquaria and feeding them abundantly as in the case of the experimental trout, for the same period of time, namely, 6 weeks. At autopsy, 7 of these trout were found to be males; all of them showed well-marked development of the testes (table 7). There was of course no way of knowing the varying states of maturation of the testes of these feeding controls, at the time they were taken from the rearing pond. Some information on their probable state of gonadal development was obtained by examining a number of trout taken from the same pond, of the same age, and approximately the same length as those shown in table 7. In 12 consecutively examined males of this group (6.2–7.4 inches, average 6.9 inches) only two exhibited developing testes with weights of 1554 and 1926 mg. while 10 had infantile testes with an average weight of 20 mg. (range, 12–38 mg.). So one can infer that some of the trout shown in table 7 had infantile testes at the beginning of the period of observation.

TABLE 7.—*Feeding controls*

[Trout taken from hatchery at 16 months of age, fed for 6 weeks, and killed in 17th month of age]

Trout number	Change in length	Increase in length of trout	Combined weight of both testes at autopsy
	Inches	*Percent*	*Mg.*
360	6.6–8.0	21	450
371	6.4–7.2	13	237
372	6.2–7.2	16	95
376	7.1–8–1	14	181
377	7.1–9.1	28	1,065
378	7.1–8.6	21	651
379	7.0–8.5	21	875
Average			508

OBSERVATIONS ON THE HISTOLOGY OF THE TESTIS

While significant increase in size of the infantile testes constitutes good presumptive evidence of beginning maturation, one cannot be certain without detailed comparative histological study that the actual cellular changes following experimental stimulation of testicular growth are the same as those occurring in the naturally maturing gonad. For this purpose a series of histological preparations were made of the testes of normal rainbow trout in all phases of maturation from the infantile to the spent gonad. The only detailed account of the histology of the testis of the rainbow trout the author has been able to find is that given by Weisel (1943) who described the immature and the mature trout's testes. No specimens of the intermediate or developing stages were studied, although he states that spermatogenesis in the rainbow trout and the sockeye salmon, which he described in their various developmental phases, are similar. The nomenclature employed in the present study for the early cytological elements in the process of spermatogenesis is the same as that used by Weisel.

Histology of the Testis in Normal Trout

The completely immature or infantile testis consists of small closely packed cysts or nests of primitive germ cells—spermatogonia [4]—which are separated into lobules comprising 2 or 3 to half a dozen or more cysts (fig. 12). The walls of the lobules, made up of connective tissue and blood vessels are often ill-defined. A few connective tissue cell nuclei are observed in the interstitial tissue as well as occasional clumps of small cells with oval nuclei. The first evidence of spermatogenesis is an increase in the number of cells in the cysts, due to division of the spermatogonia. Some of the enlarged cysts begin to exhibit a lumen (fig. 13). Frequent mitoses are seen. A few large ovoid nuclei with indistinct cytoplasm are observed in the interstitial tissues. The spermatogonia continue to divide and with increasing numbers the cells become smaller (fig. 14). At some point in this process these cells have been designated primary spermatocytes. These can readily be distinguished from the smaller spermatogonia when they exhibit nuclei densely packed with chromatin or a grouping of the chromatin material at one pole of the nucleus, the so-called synizesis stage (figs. 15, 16B, and 16C). Hann (1927) considers that synizesis marks the change from spermatogonium to primary spermatocyte; dispersion of chromatin throughout the nucleus follows. By this time the cysts have become dilated, many of the cyst walls have disappeared and the whole appearance of the gland is more lobulated with the lobules mostly oriented toward the surface of the testis.

With further division secondary spermatocytes are formed, considerably smaller than the primary ones (figs. 15 and 16D). These in turn give rise to spermatids which transform into spermatozoa without division (fig. 16E). This process is frequently focal and in its earlier phases all stages from cysts comprising only spermatogonia to lobules containing small numbers of spermatozoa may be seen. The mature testis exhibits lobules greatly distended with fully developed spermatozoa (fig. 17). Progressive transformation of the remaining immature elements continues until the only free cells are spermatozoa (fig. 18). Increase in size of the maturing testis is in general paralleled by progression of developmental stages. However, testes of equivalent size in different trout may not be in the same stage of spermatogenesis.

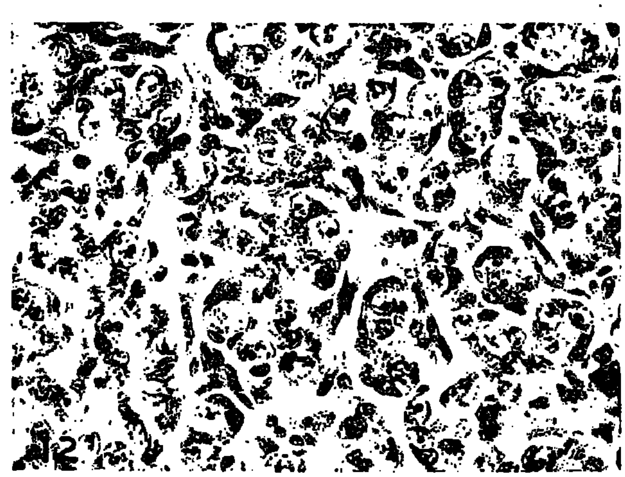

FIGURE 12.—Right testis of normal rainbow trout—length 7 to 8 inches, age 11 months. The gonad weighing 9 mg. consists of small nests of spermatogonia which in turn are grouped into lobules by septa composed of fibrous tissue and blood vessels. × 500.

[4] In a study of spermatogenesis in the Atlantic salmon parr, Jones (1940) uses the terms germ cell for the earliest stage and considers that the "germ cells" undergo a series of divisions before spermatogonia are formed. This same terminology is employed by certain other workers (Turner 1919; Bullough 1939) describing the development of the testes in other species of fish, which differs in some respects from that of the salmonids.

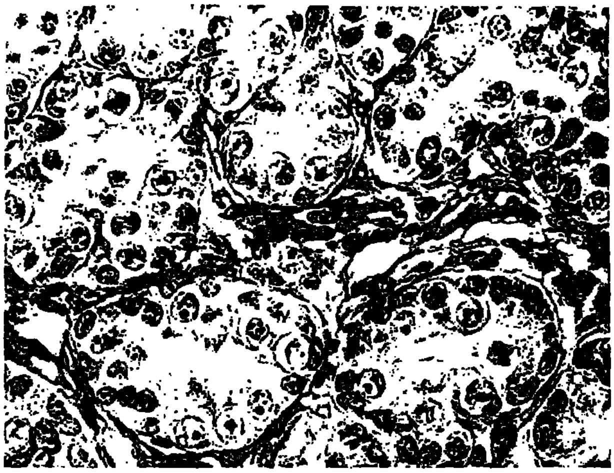

FIGURE 13.—Left testis of normal rainbow trout, length 8.4 inches, age 11 months. Testis weight 30 mg. The nests or cysts of spermatogonia show beginning enlargement with increase in the number of cells. Frequent miotic figures are evident—three occur in the photograph, two in the metaphase and one in the anaphase. × 500.

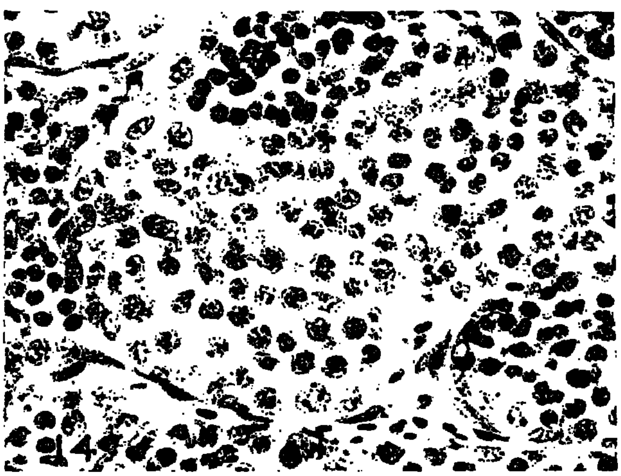

FIGURE 14.—Right testis of normal rainbow trout, length 10.4 inches, age 17 months. Testis weight 165 mg. Walls of primitive cysts have largely disappeared and cells are contained in lobules. The central lobule of the photograph consists almost entirely of spermatogonia which in the course of multiplication have diminished in size and possess little or no detectable cytoplasm. The dark staining cells in clusters above and below are primary spermatocytes. × 500.

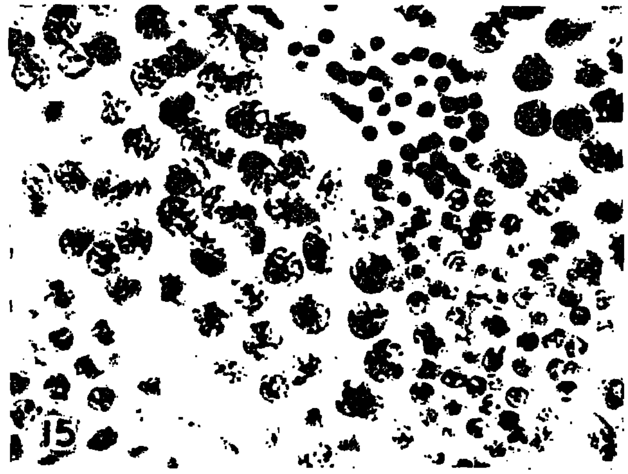

FIGURE 15.—Left testis of normal rainbow trout, length 7.9 inches, age 18 months. Spermatogenesis has proceeded further than shown in figure 14. Cells consist mostly of primary and secondary spermatocytes and small clusters of spermatids. Free spermatogonia have disappeared. × 1050.

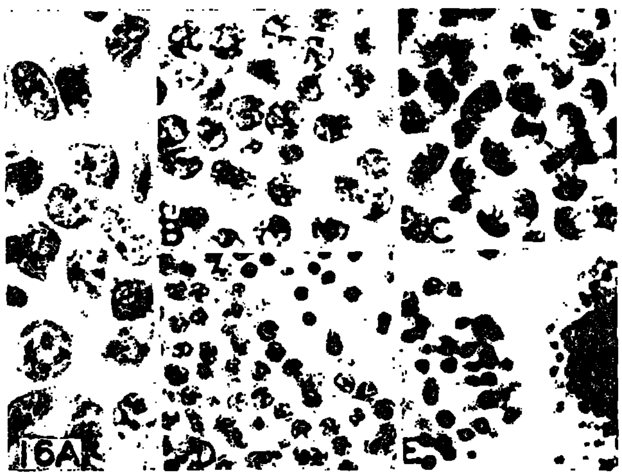

FIGURE 16.—Composite photo showing changes in size and form of cells during spermatogenesis from the free spermatogonia stage to formation of spermatozoa. (A) Spermatogonia taken from section shown in figure 14, the others from section depicted in figure 15. (B) Primary spermatocytes. (C) Primary spermatocytes in the place of synizesis. (D) Secondary spermatocytes and a few spermatids. (E) Spermatids (left) and spermatozoa (right). × 1050.

FIGURE 17.—Left testis of normal precociously mature rainbow trout; length 6.6 inches, age 8 months. Weight of testis 2,150 mg. Lobules greatly enlarged and filled with masses of fully developed spermatozoa. Cells lining walls of lobule are primary and secondary spermatocytes and spermatids. × 100.

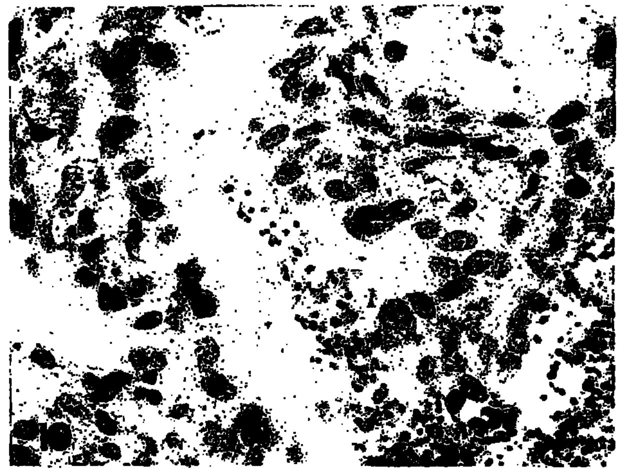

FIGURE 19.—Testis of normal spawning rainbow trout (steelhead). Testes flabby and oozing milt. Lobules much shrunken in size from fully mature state (fig. 17) and contain relatively small numbers of spermatozoa. Septa are much thickened in contrast to those in figure 18. Attached to surface of the septa are numerous lobule boundary cells—Leydig cell homologues—and an occasional spermatogonium. × 500.

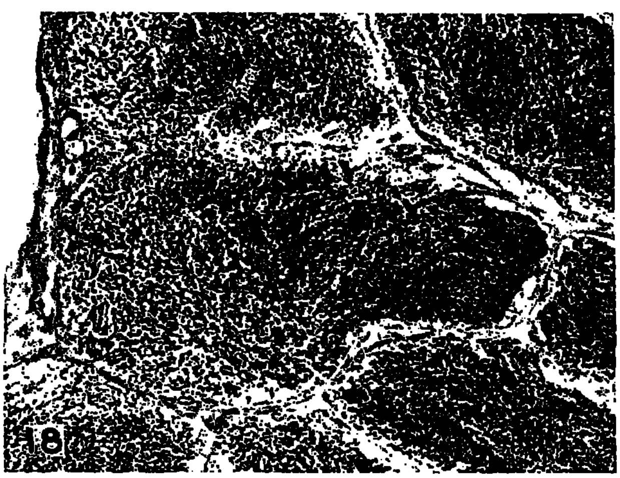

FIGURE 18.—Right testis of normal precociously mature rainbow trout; length 6.6 inches, age 11 months. Testis exuding milt partly spent, weight 453 mg. Contents of lobules consist entirely of spermatozoa; no other free cells seen. Septa thin. × 225.

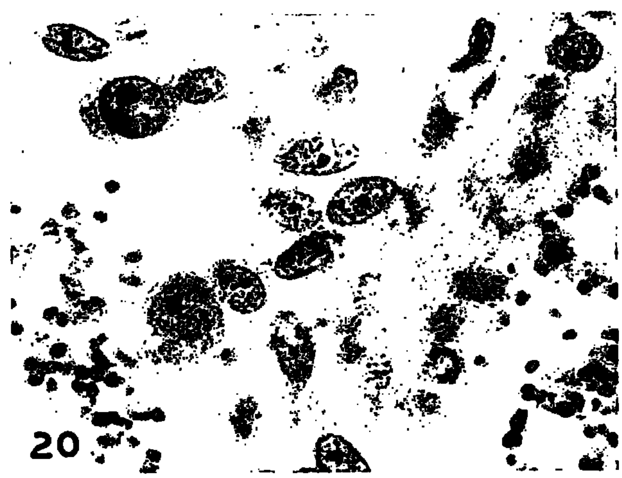

FIGURE 20.—Higher magnification of right central area of figure 19 showing several lobule boundary cells with oblong nuclei and a single nucleolus. Two spermatogonia at left of photo are easily distinguished from these cells by their larger size, rounded nucleus, which contains more heavily staining chromatin and sharply defined cell boundary. × 1050.

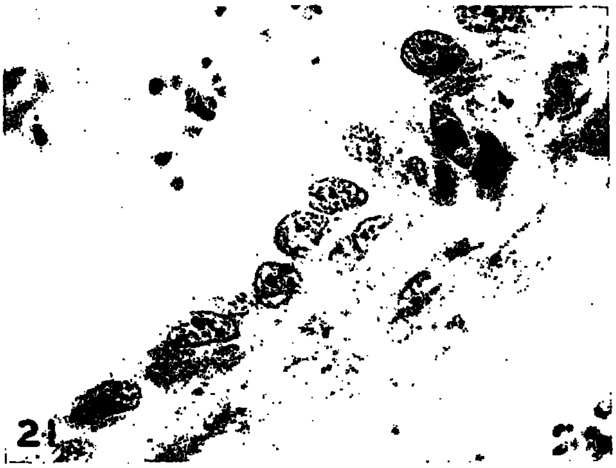

FIGURE 21.—Testis of spawning steelhead trout showing Leydig cell homologues lining the lobule. Processes of cells faintly seen. × 1050.

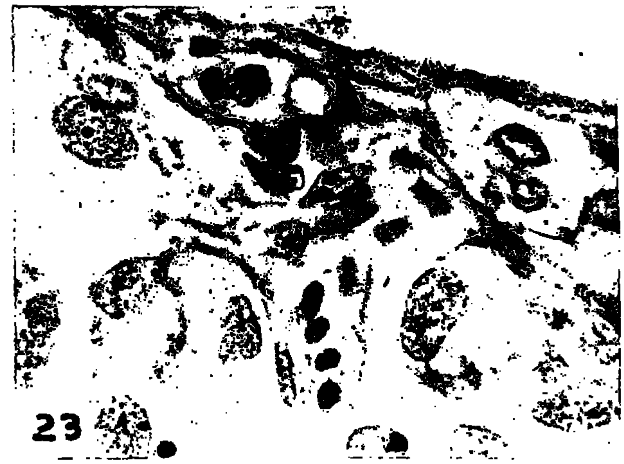

FIGURE 23.—Testis of spent steelhead trout showing Leydig-like cells in the interstitial tissues. × 1050.

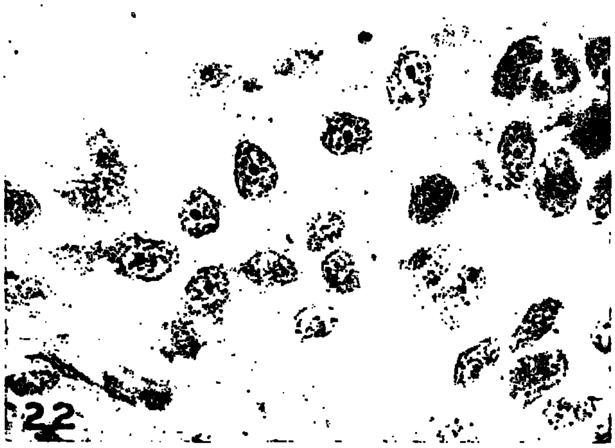

FIGURE 22.—Photo from same section as figures 19–21. In this area the lobule boundary cells are present in a syncytial-like mass. × 1050.

FIGURE 24.—Lobule boundary cells of spawning trout showing long processes extending into lumen of lobule with dark staining inclusions. × 1050.

FIGURE 25.—A group of lobule boundary cells showing inclusions. × 1050.

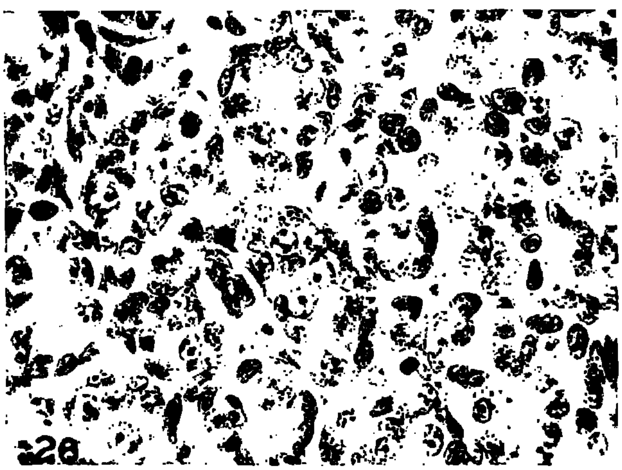

FIGURE 26.—Right testis excised from rainbow trout No. 293, length 7.2 inches, age 13 months. Weight of testis 13 mg. Histology that of infantile gonad. × 500.

With spawning, the lobules shrink and the septa become greatly thickened as shown in figure 19. Varying numbers of spermatozoa remain for some weeks. However, spermatogenesis has ceased. The lobules are lined with two quite distinct types of cell. The larger and less frequent are spermatogonia, characterized by a round nucleus containing considerable dark-staining chromatin, and a sharply defined cell boundary. The other predominant cell has an oblong nucleus with finely reticulated chromatin and a single nucleolus. Cytoplasm and cell margin is rather faint (figs. 20 and 21). They resemble the "lobule boundary cells" of Marshall and Lofts (1956) who consider them to be Leydig cell homologues. In some areas these cells are present in syncytial-like masses (fig. 22). Here and there much the same kind of cell but with a larger nucleus is present in small numbers in the interstitial tissues (fig. 23). Cytoplasmic inclusions are not uncommon in the "lobule boundary cells" and extension of the cytoplasm into tongue-like processes occurs frequently (figs. 24 and 25). Such are the chief characteristics of interstitial cells of Leydig occurring in mammals and in other groups of fishes. Failure to detect these cells in the earlier stages of the trout's spermatogenesis may be accounted for by the fact that they are probably modified connective tissue cells and quite likely do not develop until the later phase of gonadal maturation. Sertoli cells were not found.

Histology of the Testis in Trout Following Unilateral Gonadectomy

With growth in size, the remaining testes, after one was removed, exhibited the same sequence of histological changes observed in the normally maturing gonad. The only observed difference from the normal was a more focal type of early spermatogenesis occurring in many of these experimentally stimulated testes. Figure 27 shows the microscopic appearance of a remaining testis taken from a trout operated on at 13 months of age and sacrificed 6 weeks later. Primary and secondary spermatocytes as well as spermatids are present. The gonad excised at operation was found to be in the infantile state (fig. 26). With increasing size of the developing testes, the process of spermatogenesis tended to become more widespread until all the lobules were participating.

Histological examination of the testes of the control trout, i. e., those in which laparotomy and inspection only were made, revealed the finding that in fish of the same age, maturation was less advanced than in the testes remaining after unilateral gonadectomy. This was true even of the testes of control trout that showed the greatest

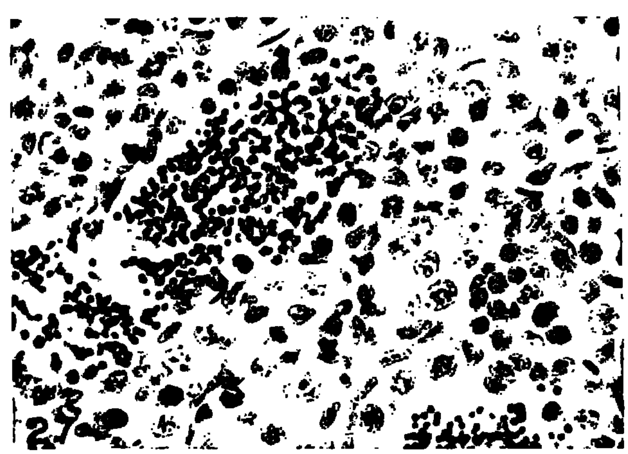

FIGURE 27.—Remaining left testis of trout No. 293 when killed weeks after unilateral gonadectomy. Testes weight 164 mg. Spermatogenesis has progressed to formation of clumps of spermatids among surrounding spermatogonia and primary spermatocytes. × 500.

growth during the experimental period. Figures 28 and 29 are photographs of the remaining testes of trout No. 332 (table 4) and of one the testis of control trout No. 336 (table 5). The percentage increase in the weight of the testis of these two fish was, as nearly as could be estimated, the same. The testis weight of the former trout, however, was 3 times that of a single testis in the latter (control) fish. In the test trout No. 332 (fig. 28), spermatogenesis is seen to be in an advanced stage with normally maturing cells (figs. 30 and 31), and although the testis of the control (fig. 29) shows all stages of development up to and including spermatozoa, these latter are present in relatively small numbers.

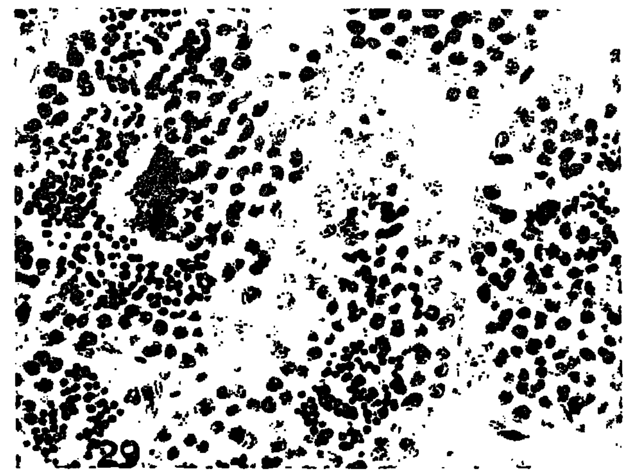

FIGURE 29.—Left testis of normal control trout, No. 336, table 5, length 7.1 inches, age 16 months, 6 weeks following laparotomy for inspection of testes which were seen to be infantile. Left testis weighed 105 mg. Histological picture that of spermatogenesis well developed to stage of spermatid formation with a few small clumps of spermatozoa as shown in photomicrograph. Process considerably less advanced than in No. 332 shown in Fig. 28. × 500.

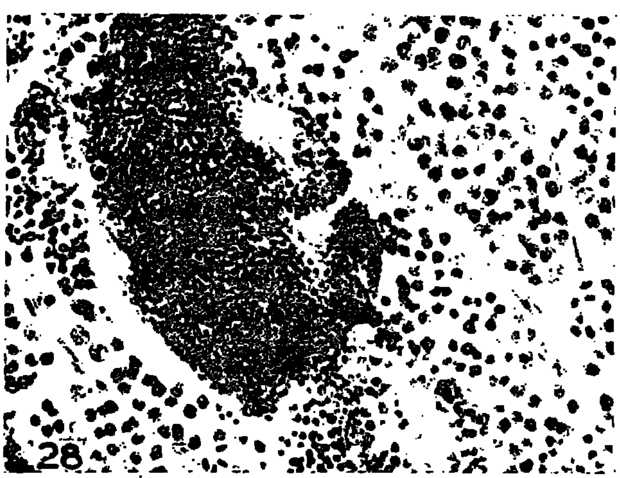

FIGURE 28.—Remaining left testis of trout No. 332, table 4, length 7.4 inches, age 16 months when killed, 6 weeks following unilateral gonadectomy. Weight of excised testis 17 mg., weight of remaining testes 335 mg. Spermatogenesis well advanced, large masses of spermatozoa present as well as spermatids, primary and secondary spermatocytes. × 500.

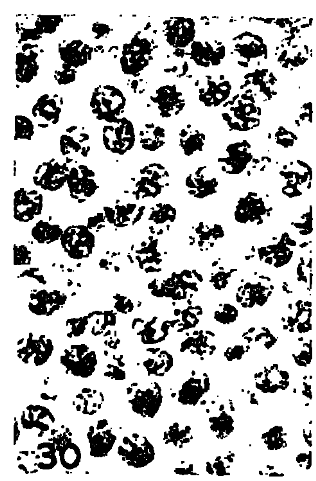

FIGURE 30.—Photographed from selected areas of section of testis of rainbow trout No. 332 shown in figure 28. Primary spermatocytes.

FIGURE 31.—Secondary spermatocytes. Cells appear to be developing normally as indicated by comparison with figures 16 (B) and (D). × 1050.

OTHER OBSERVATIONS ON STIMULATING TESTES DEVELOPMENT

The results of the foregoing experiments on unilateral gonadectomy leave unanswered a number of questions such as the reason for marked differences in response between trout of different ages, the influence of nutrition, the role played by the pituitary gland, and others. It was with the hope of obtaining more knowledge of the factors that control growth and development of the testes that the following experiments were carried out.

Depressing Effect of Starvation on the Growth of the Testes

Rate of growth of the individual experimental fish bore no relation to the amount of weight gain of the testes, either in the unilaterally gonadectomized trout or in the controls. While the maximum increase in the weight of the testes observed in a given experiment most often occurred in those trout exhibiting the greatest gain in length, not infrequently a moderate increase in the size of the trout was associated with a greater percentage gain in testis weight than occurred in a fish showing twice as much growth. However, in those few trout showing relatively little growth (not more than 2 or 3 percent during the period of the experiment) there was no significant increase in the weight of their testes. This led to testing the effect of starvation.

A number of trout from which one testis had been removed were starved for 25 days. The results are shown in table 8. With 4 exceptions, the remaining testes in these starved fish exhibited little or no gain in weight, despite the fact that most of the fish were in an age group and were operated on at a time of year when marked growth of the remaining testis occurred in fed fish. (See table 1.) However, the trout exhibited in table 8A cannot be compared quantitatively with those shown in table 1, since the period of observation is considerably shorter in the former group. A second similar experiment was carried out in which the fish were starved for 35 days. The results (table 8B) were essentially similar to those in the trout starved for 25 days. The average gain in weight of the remaining testes of the group was less than 60 percent, a negligible amount.

TABLE 8.—*Depressing effect of starvation on growth of remaining testis following unilateral gonadectomy*

Trout No.	Length of trout	Age at autopsy	Weight of excised testes	Estimated weight of remaining testes	Remaining testis at autopsy			Change in length of trout
					Weight	Gain in weight	Gain in weight	
A. Starvation period 25 days	*Inches*	*Month*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Percent*	*Inches*
65	7.8	12	R 10	L 12	25	13	108	0
66	8.2	12	L 9	R 7	15	8	114	−0.1
86	7.9	12	L 12	R 10	11	1	10	−0.1
87	7.8	12	R 12	L 14	22	8	57	0
113	6.5	15	L 52	R 44	46	2	4	−0.1
118	8.1	15	R 9	L 10	22	12	120	0
124	8.0	15	L 14	R 12	45	33	275	0
181	8.0	19	L 8	R 7	6	−1	−14	−0.2
174	6.0	19	L 10	R 9	8	−1	−11	−0.2
176	7.7	19	L 13	R 11	18	7	60	−0.1
178	7.6	19	L 20	R 18	15	−3	−16	−0.1
419	8.6	19	L 18	R 15	20	5	33	0
420	8.0	19	R 10	L 12	18	6	50	+0.1
Average							59	
B. Starvation period 35 days								
464 [1]	8.0	22	L 11	R 9	10	1	10	−0.1
462	7.7	23	L 13	R 11	19	8	73	0
467	8.3	23	L 13	R 11	14	3	27	0
469	7.0	23	R 7	L 8	12	4	50	0
473	8.9	23	L 21	R 18	36	18	100	0
Average							52	

[1] 28 days' starvation.

NOTE.—The average loss in body weight of starved trout equaled 8 percent.

Stimulability of the Testes in Young Trout

The relatively small increase in weight of the remaining testes of the young unilaterally gonadectomized trout as compared with that observed in the older trout similarly treated (see table 3) could be attributed either to lack of sensitivity of the testes to gonadotrophic action or to failure of the juvenile pituitary to produce gonadotrophins in response to removal of one gonad. Test of the reactivity of the infantile male gonad in 7- to 9-month-old trout was made by implanting, intramuscularly, pituitaries taken freshly from trout with actively developing testes as well as from fish with infantile gonads. The testes of the recipient trout were inspected just before implantation of the pituitaries. The donors of the pituitaries ranged from 7 to 20 months of age. The implants were made into a pocket excavated in the muscle of the caudal peduncle. Cutting the pituitaries in 2 parts appeared to have no advantage over implanting them whole. Closure of the wound was made with closely spaced sutures. At the termination of the observation period, 35 days, the implants were examined under the dissecting microscope and if found were placed in Bouin's solution for later histological examination. The details of these experiments and the results are shown in table 9.

Table 9.—*Effect on testicular development in young immature trout 7 to 9 months old, of intramuscular implantation of pituitaries from male fish with developing or immature gonads*

[Period of observation 35 days]

Recipient trout No.	Donors of pituitaries			Survival of implants	Recipient's testes at autopsy		
	Age months	Weight of testes (mg.)	Degree [1] of testes development		Weight (mg.)	Percent gain in weight	Histological evidence of maturation [2]
185	[3] 20	4,800	+++	+	3,486	16,778	+
	7	2,500					
188	20	2,500	+++	+	749	4,680	+
	20	2,200					
201	20	2,590	+++	+	139	1,042	+
	20	2,500					
228	9	1,360	++	+	229	1,250	+
227	9	1,680	++	0	50	230	0
277	23	222	+	0	34	0	—
226	9	105	+	0	76	170	±
215	9	29	0	+	21	30	0
223	9	39	0	+	36	56	0
224	9	31	0	+	27	66	0

[1] Degree of testes development in pituitary donors:
+++=approaching full maturity.
++=well marked development.
+=beginning development.
0=infantile testes.

[2] Histological evidence of maturation:
+=well marked.
±=beginning.
0=none.
—=not done.

[3] When 2 pituitaries were implanted at a time 1 of them was cut in half beforehand. In single pituitary implants some were divided, others used whole.

It will be noted that 4 out of 7 trout receiving pituitaries from donors with large testes showed well-marked testicular growth. In one, R. T. 185, the testes grew from an estimated 25 mg. (observed at operation) to 3,486 mg. within 5 weeks. Figure 32 exhibits a photograph of these testes

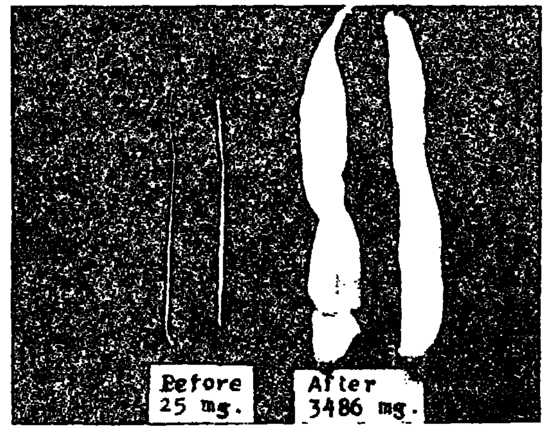

Figure 32.—Testes (right side of photo) of trout No. 185, table 9, 5 weeks following implantation of 2 pituitaries taken from rainbow trout with maturing testes. The testes on the left of the photo, excised from another fish, were of the same estimated size and appearance as the testes of No. 185.

removed at autopsy together with a pair of 25-mg. testes taken from another trout for comparison. In three cases Nos. 226, 227, and 277, no living pituitary tissue was found on microscopical examination of the implant, and little or no growth of the recipient's testes occurred in these fish. In trout Nos. 215, 223, and 224, implants of pituitaries taken from donors with immature testes showed well-organized and vascularized pituitary tissue but failed to produce any significant growth or maturation of the recipient's gonads.

Effects of Testis Implants in Immature Trout

In an attempt to determine what effect the implantation of immature testicular tissue would have on the growth of the remaining intact gonad, a series of experiments were undertaken in which one infantile testis was excised and immediately implanted into the spleen, or the peritoneal cavity, or into a muscle pocket in the caudal peduncle. The grafts took in all these sites. However, the relation of this procedure to increased growth of the remaining intact testis was equivocal. Of the first 4 trout thus treated, 2 showed at the end of 6 weeks moderate to very marked accelerated growth of the remaining testis. The other 2 exhibited no change in size of the intact gonad. These trout were at an age and time of year when spontaneous gonadal development was very apt to occur. The next 5 trout treated in this way were 7 to 8 months of age when only a small percentage of male trout normally show precocious sexual development. Four of the 5 grafts were found to be living. In one of these 4, the remaining testes exhibited well-marked development with an increase in weight of 600 percent over its estimated size at the beginning of the experiment. The other 3 showed no more weight increase of the remaining gonad than one might expect from the effect of laparotomy alone. Evidence of spermatogenesis in the implanted testicular tissue was found only in those trout showing accelerated growth of the remaining testis, which suggests spontaneous increased pituitary activity in these instances. So the results of the foregoing experiments provide no evidence for either suppression or acceleration of testis growth associated with implants of the trout's own testicular tissue.

Implants of infantile or maturing testes taken from other trout failed to survive.

Rate of Growth and Development of Testes Under Experimental Stimulation

The findings of the present study indicate that the immature testes of the rainbow trout can grow with extraordinary rapidity when fish are at the appropriate age and season of the year. Table 2 displays a number of instances of this kind. In unilaterally gonadectomized fish, increases in the weight of the remaining testis of 400- to 500-fold within 6 weeks were not uncommon in trout 17 to 18 months of age. Figure 33 illustrates an instance of this kind. Whether such rapid growth occurs in rainbow trout under normal conditions is not known, since laparotomy for inspection is essential to determine the size of the testes at the beginning of the observation. And it may well be that the stress resulting from the operative procedure is responsible to a considerable degree for the intense pituitary activity. However, it seems probable from the data assembled in the present study, that when optimum conditions occur, growth and development of the infantile testis in the normal rainbow trout can take place in a relatively short space of time.

None of the considerable number of experimental fish studied had reached full gonadal maturity, even those kept under observation for as long as 10 weeks. Microscopic examination of the largest and most grossly mature appearing testes showed the predominant cell type to be spermatocytes with, however, many spermatids and spermatozoa; but the latter were not present in the great masses found in the fully ripe testes.

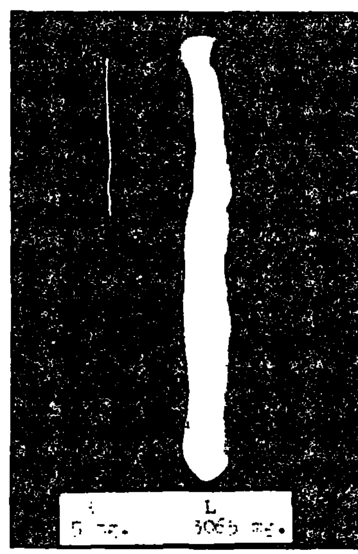

FIGURE 33.—Testes of trout No. 151, 6.1 inches long and 17 months of age when right testis was excised. Six weeks later when trout was killed, left testis weighed 3,065 mg. a 509-fold increase in weight.

DISCUSSION

The literature on unilateral gonadectomy in fishes is exceedingly sparse. Only one reference was found in which the effect of the procedure on the remaining testis was mentioned.[5] Craig-Bennet (1931) observed some hypertrophy of the remaining testis of the unilaterally gonadectomized 3-spined stickleback but gives no weights. Tozawa (1929) performed unilateral gonadectomies on the bitterling but made no mention of change in size of the testes remaining. On the other hand, a number of studies on this subject have been made with birds and mammals. Domm and Juhn (1927) found that the nonexcised testis of the chicken, when examined 32 weeks after

[5] See Addenda.

unilateral gonadectomy, weighed as much as the 2 testes of controls of similar age and size. They called this phenomenon compensatory hypertrophy. Belsky (1936) also working with chickens observed hypertrophy of the remaining testis but only some 25 to 30 percent greater than normal. Observations on the effect of unilateral gonadectomy in mammals goes back to Ribbert (1895) who employed adult rabbits and guinea pigs. He found that after an interval of 7 months the remaining testis of operated rabbits weighed almost twice as much as a single testis of the controls. The same effect was observed in guinea pigs but the weight of the nonexcised testis was only 40–50 percent greater than that of a single gland of the control. Lipschutz's (1922) results in young rabbits confirmed those of Ribbert. Likewise, Saller (1933) found that old mice responded to unilateral gonadectomy by a doubling of the weight of the remaining testis.

The results of the present study indicate that under the optimum experimental conditions, the remaining testis in the unilaterally gonadectomized trout may increase to 4 times the size of a single testis in the control during a period of 6 weeks (see table 3). This is twice the maximum growth of the remaining gonad recorded for mammals and birds observed over much longer periods of time. However, just below and above the optimum age of 16 months the weight of the remaining testis in the trout was approximately equal to the combined weights of the 2 testes in the controls.

The principal difficulty in demonstrating this phenomenon in the rainbow trout is that the operative procedure of simply opening and closing the peritoneal cavity appears to stimulate the initiation of growth and development of the testes. The effect is slight in young trout up to 14 months of age but increases progressively after this age until at 18 months the testis growth of the operated controls may be just as rapid as that of the unilaterally gonadectomized fish. It seems probable that the stress of operation constitutes the stimulating factor.

The lack of any detectable effect of unilateral gonadectomy in trout younger than 13 months of age and the significant and progressive percentage gain in weight of the remaining testis with increasing age (up to 17 months) suggests that the pituitary of the juvenile trout fails to respond to the stimulus of removing one gonad. That the infantile testis is fully responsive to pituitary gonadotrophins was shown by the very rapid growth of the testis in 7- to 9-months-old fish implanted with pituitaries taken from other trout exhibiting maturing testes. Further and equally clear evidence for stimulability of the juvenile testes is the occurrence of spontaneous precocious maturity in some 10 percent of rainbow trout at this age. These facts imply that in the great majority of fish, the ability of the pituitary to produce gonadotrophins is very limited during the first year of the rainbow trout's life. The lack of any demonstrable gonad-stimulating effect of implanted pituitaries from young trout with infantile testes (table 9, trout Nos. 215, 223, 224) supports this inference. With the beginning of the second year the gonadotrophic function develops progressively until at about a year and a half the pituitary is capable of producing large amounts of the hormone. Furthermore, with increasing age the pituitary becomes more easily stimulated, as shown by the greater effects on the testes of both unilateral gonadectomy and laparotomy alone. Most striking in this respect is the fact that just bringing trout into the laboratory and feeding them well with consequent rapid growth, caused a pronounced testicular development in all the males so treated (table 7). It should be pointed out that increased growth rate resulting from abundant food supply may not be the only factor involved in changing the habitat of the trout from outdoors to indoors. Diminished light and close confinement are possible influences in this respect, but of undetermined importance in the present experiments.

Whether the accelerated growth and development of the testis remaining after unilateral gonadectomy is due entirely to increased output of gonadotrophin from the pituitary is not clear. Weiss (1947) brought forward evidence to show that partial removal of an organ will automatically entail compensatory growth of the rest of the organ by means of reducing the concentration of certain diffusible components from the organ itself which are capable of limiting its growth. Attempts to study this possibility through reimplanting the excised testis gave equivocal results. However, improved techniques of tissue transplantation might yield more information. Evidence for this phenomenon being predominantly, if not entirely, a gonadotrophin effect is provided by the action of pituitary implants as shown in table 9. Accelerated growth of the recipient's testes occurred only when the implanted pituitaries came from donors with developing gonads and only when the implants survived. Unilateral gonadectomy in hypophysectomized trout would provide another analytical approach.

The present observations on the histological changes occurring in the testes of the rainbow trout are in agreement with those described by Weisel (1943) except for the occurrence of cells homologous with the interstitial cells of Leydig. Weisel stated that in neither the rainbow trout nor the sockeye salmon did he detect in the interstitial tissues any cells which had the appearance of secretory function. More recently, Potter and Hoar (1954) have reported the presence of interstitial cells in the testis of Pacific salmon (*O. keta*) which appear to resemble closely Leydig cells of the mammalian male gonad. Marshall and Lofts (1956) were the first to describe the occurrence of Leydig cell homologues lining the lobules in the testes of certain teleost fishes, including the char (*Salvelinus willughbii*). They have designated these as "lobule boundary cells" in contradistinction to the interstitial cells of Leydig and believe that they evolve from fibroblasts present in the lobule septa. Miller and Robbins (1954) had previously described the presence of similar cells lining the lobule of the spent testis in the salamander *Taricha torosa*. They stated that "the tissue analagous to the interstitial cells of the higher vertebrates consists of a layer of lipid containing cells of connective tissue origin together with a layer of transformed Sertoli cells." The findings of the present study, namely the occurrence of both lobule boundary cells and Leydig-like cells in the interstitial tissues would seem to place the rainbow trout in a category distinct from those fishes which exhibit only one of these two cell types. (See Marshall and Lofts.)

From accounts in the literature the process of spermatogenesis in the salmon appears to be quite similar to that observed in rainbow trout except for possible differences in the earliest stages. Jones (1940) describes migration of the germ cells in the maturing Atlantic salmon parr. Weisel makes no mention of any such phenomenon in the early maturation of the adult land-locked sockeye salmon. Certainly there was no evidence of migration of germ cells or spermatogonia in the rainbow trout of the present investigation. Further study of other species of salmonids should help clarify this subject. The reader is referred to Turner's (1919) account of spermatogenesis in the perch for the description of a special type of migration of germ cells.

The occurrence of precocious sexual maturity in the trout observed in the present work is apparently quite a common phenomenon in the Atlantic salmon. A study by Orten, Jones, and King (1938) summarizes earlier data on this subject and adds their own observations. They found that about 40 percent of the population of salmon parr captured in the fall of the year were sexually mature. They also make the statement that precocious sexual maturity occurs notably in rainbow trout.

SUMMARY

An operative procedure for unilateral gonadectomy was developed and carried out in rainbow trout with a very low resulting mortality. Removal of one gonad from male trout with infantile testes at a suitable age, stimulated the initiation of spermatogenesis in the remaining gonad which showed rapid growth and development. Control trout of the same size and age in which laparotomy only was performed, exhibited similar changes but to a much lesser extent.

Unilateral gonadectomy in trout under one year of age resulted in relatively little increase in the size of the remaining testis, while from one year on, the effect of this procedure on testes growth increased progressively. That this difference was not due to the lack of sensitivity of the young immature testis to gonadotrophic action was indicated by the marked growth and development of such testes following intramuscular implants of pituitaries taken from trout with maturing testes. Rather, it seemed probable that the pituitary of the young sexually immature fish was incapable of responding to the stimulus of unilateral gonadectomy. This inference was supported by the absence of demonstrable gonadotrophin content of the pituitaries of such fish. Starvation suppressed the growth of the testes in both unilaterally gonadectomized and control trout.

Observations were made on the histological changes occurring during the complete cycle of spermatogenesis in both normal and experimental trout. No significant differences between the two were found. Also data on the size and growth rate of the testes of normal rainbow trout and the incidence of naturally occurring precocious maturity in young males are presented.

REFERENCES

BELSKY, N. W.
1936. Die analyse der hypertrophieerscheinung der gonaden bei einseiteger kastration der vogel. Ztschr. f. Vergleich. Physiol. 23: 578–591.

BULLOUGH, W. S.
1939. A study of the reproductive cycle of the minnow in relation to the environment. Zool. Soc. Proc. Ser. A 109: 79–102.

CRAIG-BENNETT, A.
1931. The reproductive cycle of the three-spined stickleback (*Gasterosteus aculeatus*, Linn.) Philosoph. Trans. Royal Soc. London 299B: 197–278.

DOMM, L. V. and M. JUHN.
1927. Compensatory hypertrophy of the testes in brown leghorns. Biol. Bull. 52: 458–473.

HANN, H. W.
1927. The history of the germ cells of *Cottus bairdii* Girard J. Morph. 43: 427–497.

JONES, J. W.
1940. Histological changes in the testes in the sexual cycle of the male salmon parr (*Salmo salar* L. juv.). Proc. Royal. Soc. London Ser. B 128: 499–509.

LIPSCHUTZ, A.
1922. The so-called compensatory hypertrophy of the testicle after unilateral castration. J. Physiol. 56: 451–458.

MARSHALL, A. J. and B. LOFTS.
1956. The Leydig-cell homologue in certain teleost fishes. Nature 177: 704–705.

MILLER, M. R. and M. E. ROBBINS.
1954. The reproductive cycle in *Taricha torosa*. (*Triturus torosus*). J. Exp. Zool. 125: 415–446.

ORTON, J. H., J. W. JONES, and G. M. KING.
1938. The male sexual stage in salmon parr (*Salmo salar* L. juv.). Proc. Royal Soc. London Ser. B 125: 103–114.

PHILLIPS, A. M., JR., F. E. LOVELACE, D. R. BROCKWAY' and G. C. BALZER, Jr.
1953. The nutrition of trout. Fisheries Research Bull. No. 17, Cortland Hatchery Report No. 22. N. Y.

POTTER, G. D. and W. S. HOAR.
1954. The presence of androgens in chum salmon (*Oncorhynchus keta* Walbaum). J. Fish. Res. Bd. Canada. 11: 63–68.

RIBBERT, H.
1895. Beiträge zur kompensatorischen hypertrophie und zur regeneration. Arch. Entwickel, mech. d. organ. 1: 69–90.

ROBERTSON, O. H., and A. P. RINFRET.
1957. Maturation of the infantile testes in rainbow trout (*Salmo gairdnerii*) produced by salmon pituitary gonadotrophins administered in cholesterol pellets. Endocrinology 60; 559–562.

SALLER, K.
1944. Über den einfluss einseitiger kastration (halb kastration) auf die zurückbleibende keimdrüse (Nach untersuchungen an männlichen weissen hausmäusen). Roux Arch. f. Entwickel, mech. d. organ. 129: 410–441.

TOZAWA, T.
1929. Experiments on the development of the nuptial coloration and pearl organs of the Japanese bitterling. Folia Anat. Japonica 7: 407–417.

TURNER, C. L.
1919. The seasonal cycle in the spermary of the perch. J. Morph. 32: 681–711.

WEISEL, G. F.
1943. A histological study of the testes of the sockeye salmon (*Oncorhynchus nerka*). J. Morph. 73: 207–229.

WEISS, P.
1947. The problem of specificity in growth and development. Yale J. Biol. and Med. 19: 235–278.

ADDENDA

1. Several additional references in unilateral gonadectomy in fishes have recently come to the author's attention:

Bock, Friedrich.
1928. Kastration und sekundäre geschlechtsmerkmale bei teleostiern. Ztschr. f. Wiss. Zool. 130: 455–468.

Bock noted increase in size of remaining testis in unilaterally castrated sticklebacks, but gave no weights.

Peters, Hans M.
1957. Über die regulation der gelegrösse bei fischen. Ztschr. f. Naturforsch. 12-b: 255–261.

Peters observed that after removal of one ovary or parts of both glands the remaining ovarian tissue regenerated to the point of producing the normal number of eggs and mass of egg tissue but not in excess of normal.

Wunder, Wilhelm.
1955. Das verhalten von hoden, niere und leber nach entfernung von teilstücken beim karpfen. Zool. Anz. 155: 232–239.

Wunder found an increase in the weight of the remaining testis threefold to sixfold that of the excised gland. He employed carp with well-developed testes and gives no adequate operative controls; hence his results are in no way comparable with those of the present study.

2. Fixation of the testes of spawning steelhead trout revealed an intense concentration of lipid material in the lobule boundary cells, thus providing additional evidence for their functional identification with the Zeidig interstitial cells of the mammalian testis.

U. S. GOVERNMENT PRINTING OFFICE: 1958 O—435916