*CHANGES IN PLASMA 17-HYDROXYCORTICOSTEROIDS ACCOMPANYING SEXUAL MATURATION AND SPAWNING OF THE PACIFIC SALMON (ONCORHYNCHUS TSCHAWYTSCHA) AND RAINBOW TROUT (SALMO GAIRDNERII)**

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In the course of an investigation into the nature of the post-spawning death of the Pacific salmon,[1-3] marked hyperplasia of the adrenal cortical tissue was found to be present constantly in the sexually mature fish of both sexes.[4, 5] Beginning hyperplasia was observed in immature salmon taken on their spawning migration several months before they were due to spawn. This change became more pronounced with progressive development of the gonads. Studies on the anadromous form of the rainbow trout, the steelhead, which suffer a considerable mortality after spawning, also revealed the presence of marked hyperplasia of adrenal cortical tissue at full sexual maturity, whereas the nonmigratory rainbow trout (hatchery reared) which usually survive their initial reproductive effort showed very little hyperplasia of the adrenal tissue.

The present study was undertaken to determine the presence of adrenal corticoids in the blood plasma of salmon at various stages of their spawning migration and sexual development, as well as in fully mature rainbow trout, migratory and nonmigratory.

Material and Methods.—Source of fish: Twenty-two king salmon in their second, third, and fourth years of age were caught by hook and line in the sea in Monterey Bay in August, 1957, and just off the entrance of Bolinas Bay north of San Francisco in July, 1958. These fish migrate up the Sacramento and San Joaquin Rivers. Their gonads were in an infantile state, so that it was not possible to predict exactly how close they were to starting their spawning migration.

Fourteen male salmon were taken in wire traps on the Sacramento River in September, 1958, at a point designated as Fremont, about 125 miles from the sea. These were fall-run fish which began their fluvial migration from August to October with gonads already developing. With three exceptions they exhibited gonads in an advanced state of maturation and were probably due to spawn within 6 weeks to 2 months. Fourteen salmon were secured at Mill Creek, a tributary of the Sacramento River, approximately 285 miles up stream, in May, 1958. These were all spring run, i.e., those which leave the sea from March to May and, with two exceptions, their gonads were in a relatively early stage of development. The expectation was that they would not spawn for 4 to 5 months. They were taken in a trap at the top of a ladder on the Mill Creek Dam.

Spawning salmon were trapped at the Coleman Hatchery on Battle Creek, another branch of the Sacramento River, about 325 miles from the sea, October to December 1957, 1958. The Coleman salmon are fall-run fish. Some of these, after being artificially spawned, were held for 3 or 4 days before blood samples were drawn. Fifty ripe and spent (held) fish were studied.

Twelve king (Chinook) salmon were secured at the Rock Island Dam on the Columbia River some 500 miles up stream. These, all males in their second year (grilse), exhibited testes in a relatively early state of maturation.

Twenty-seven steelhead trout ready to spawn were caught in traps in the Eel River at two points 82 and 154 miles from the sea.† Seven sexually mature males and 25 immature nonmigratory rainbow trout were obtained from the hatchery of George Dufour, Santa Cruz, whom we wish to thank.

Bleeding: The fish were lightly anesthesized beforehand in a 1–25,000 solution of tricaine-

methanesulfonate (Sandoz) which was kept well aerated by means of an air pump and gas dispersers. Bleeding was performed by heart puncture using heparin as an anticoagulant. The blood sample was immediately put on ice and centrifuged. The plasma was drawn off and iced until the samples could be deep-frozen.

Determination of free plasma 17-hydroxycorticoids (17-OHCS): Plasma 17-OHCS were determined by the methods of Peterson and Wyngaarden, *et al.*[6] and Silber and Porter.[7] The Allen correction principle[8] for maximum absorption was applied.

Identification of the adrenal corticoids: The salmon plasma was extracted with methylene dichloride and chromatographed on paper, with standards in parallel, by Zaffaroni's method,[9] using chloroform saturated with formamide. The areas corresponding to hydrocortisone and cortisone and the area from cortisone to the front were eluted and rerun in the Bush (B_4) system.[10] Standards in each case were run in parallel.

Identification of the steroids were based on (*a*) photographs in ultraviolet light (λ 235.7 mμ), (*b*) reaction with "blue tetrazolium," (*c*) Rf values of substances compared with standards run in parallel, and (*d*) absorption spectra of sulfuric acid chromogens.[11]

RESULTS

Plasma Concentration of 17-Hydroxycorticosteroids.—(1) Sea salmon: Since only relatively small quantities of blood could be obtained from the fish caught by hook and line, it was necessary to pool the samples of plasma in order to obtain sufficient material for corticoid determination. Table 1 lists 17-OHCS values for two pools

TABLE 1

BLOOD LEVELS OF 17-HYDROXYCORTICOIDS (μG/100 ML) IN SEA SALMON

Date	Location	No. of Fish	Plasma
August, 1957	Monterey Bay	Pool of 4 fish	10.6
July, 1958	Bolinos Bay	Pool of 7 fish	13.2
July, 1958	Bolinos Bay	1 female	7.0

and a single fish, giving a mean concentration of 11.8 μg/100 ml. No distinction between sexes was made in the pools.[12]

(2) Migrating salmon with maturing gonads: Individual plasma corticoid determinations were made on 7 male and 7 female salmon secured at Mill Creek. Table 2 shows the results of the determinations. There was a very slight dif-

TABLE 2

BLOOD LEVELS OF 17-HYDROXYCORTICOIDS (μG/100 ML) IN SALMON ON SPAWNING MIGRATION

Fremont* Males	Mill Creek‡ Males	Mill Creek‡ Females
50.0	20.3	34.8
33.4	62.7	57.2
62.0	46.5	51.7
27.5	58.5	44.8
47.8	24.4	77.1
36.3	75.5	60.0
32.5	53.4	47.9
Mean 41.4 ± 12	49.0 ± 21	53.4 ± 13

* Fall run. ‡ Spring run.

ference between the Mill Creek males and females, namely 49.0 ± 21 μg/100 ml for the former and 53.4 ± 13 μg/100 ml for the latter. The Mill Creek males with immature testes exhibited a little higher 17-OHCS level than the Fremont males whose gonads were in an advanced stage of maturation: 49.0 ± 21 μg/100 ml and 41.4 ± 12 μg/100 ml, respectively. Twelve salmon (grilse) with immature

gonads taken at Rock Island Dam on the Columbia River had an average level of 89 μg/100 ml.

(3) *Spawning and spent (held) salmon:* Plasma 17-OHCS levels were determined in a total of 24 males and 26 females obtained at the Coleman Hatchery. Fourteen spawning and spent fish in 1957 had mean corticoid levels of 28.4 μg/100 ml for the males and 81.2 μg/100 ml for the females. Thirty-six fish in 1958 showed mean values of 32.4 ± 13 μg/100 ml for the males and 77.4 ± 28 μg/100 ml for the females. No significant differences were found between ripe and spent fishes.

TABLE 3

BLOOD LEVELS OF 17-HYDROXYCORTICOIDS (μG/100 ML) IN SPAWNING AND SPENT SALMON

1957		1958	
Males	Females	Males	Females
26.6*	113.0*	52.1	80.0
29.2*	47.4*	19.2†	59.1
42.1*	102.0*	37.1	48.0†
16.0*		37.1	50.9
		45.9	101.0
		13.5	62.6
		11.7	74.6
		46.6	83.1
		32.5	134.0
		11.7	54.7
		46.6	57.5
		32.5	69.3
		44.3	77.1
		14.6	74.5
		24.2	72.0
		37.5	59.1
		42.6	78.9
		27.8	158.0
Mean 29.0 ± 11	88.5 ± 35	32.4 ± 13	77.4 ± 28
27.3 (2)‡	77.5 (5)‡		

* Artificially spawned 4 days previously.
† Artificially spawned 3 days previously.
‡ Pooled plasma. Figure in parentheses = number of fish.

Corticoid values are shown in Table 3. Three spawning grilse taken at Coleman Hatchery exhibited a corticoid concentration of 29.6 μg/100 ml.

(4) *Spawning steelhead trout:* Plasma 17-OHCS levels were determined in 13 males and 14 females (Table 4). Six individual males had a mean value of 21.6 ± 5 μg/100 ml and values of 26.2 and 23.2 μg/100 ml on 2 and 5 pooled plasmas,

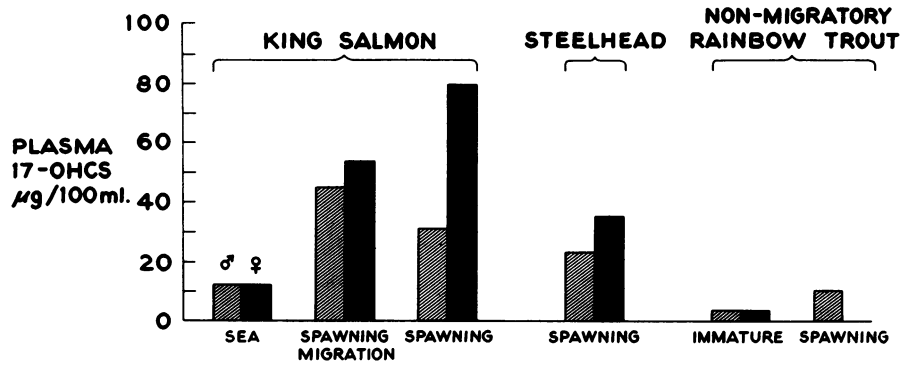

FIG. 1.—Plasma 17-OHCS levels with sexual maturation and spawning.

TABLE 4

BLOOD LEVELS OF 17-HYDROXYCORTICOIDS (μG/100 ML) IN SPAWNING STEELHEAD TROUT

	Males	Females
	26.4	30.6
	19.8	31.1
	16.0	32.1
	26.4	17.1
	16.0	55.1
	25.2	23.4
		24.1
		23.2
		41.5
		68.3
		26.8
		36.2
Mean	21.6 ± 5	34.1 ± 15
	26.2 (2)*	36.5 (2)*
	23.2 (5)*	

* Pooled plasma. Figure in parentheses = number of fish.

respectively. Twelve females had a mean value of 34.1 ± 15 μg/100 ml and 36.5 μg/100 ml. on a plasma pool of 2 fish.

(5) *Sexually immature and mature nonmigratory rainbow trout:* Heparinized blood was obtained by severing the tail of the trout. Pool plasma from 25 im-

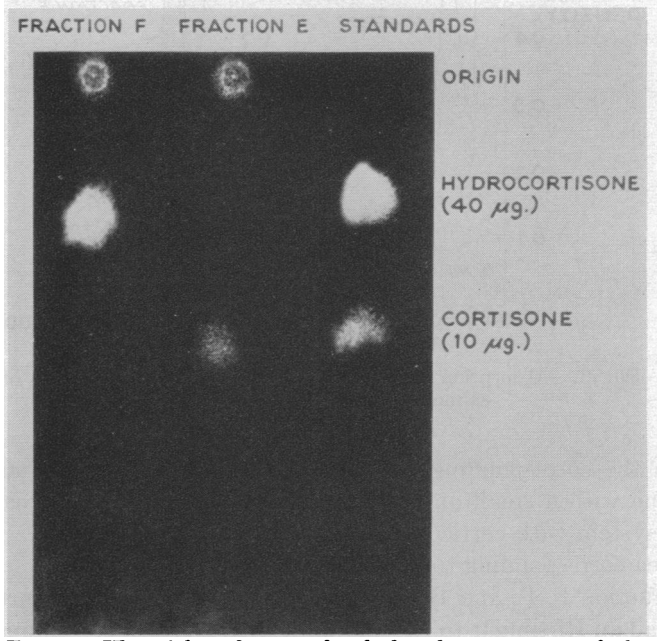

FIG. 2.—Ultraviolet photograph of the chromatogram of the salmon F and salmon E fractions.

mature males and females and a pool from 7 mature males showed 17-OHCS concentrations of 2.7 and 10.0 μg/100 ml, respectively.

Changes in the 17-OHCS levels with sexual maturation and spawning in both the males and females are summarized in Figure 1. Comparison is also shown

between the spawning king salmon, steelhead trout and nonmigratory rainbow trout.

Nature of the Adrenal Corticoids.—One hundred ml of pooled plasma from spawning female salmon containing 77.5 μg of 17-OHCS was extracted and subjected to paper chromatography in the Zaffaroni's chloroform-formamide system in an endeavor to identify the adrenal corticoids present. The areas corresponding to Compounds F, E (fractions F and E, respectively) and the area below E to the front (fraction B) were sectioned and eluted. Areas of fraction F and E from the first chromatogram were rerun on paper in Bush's toluene-methanol-water system. Figure 2 shows a photograph in ultraviolet light with fraction F and fraction E running in

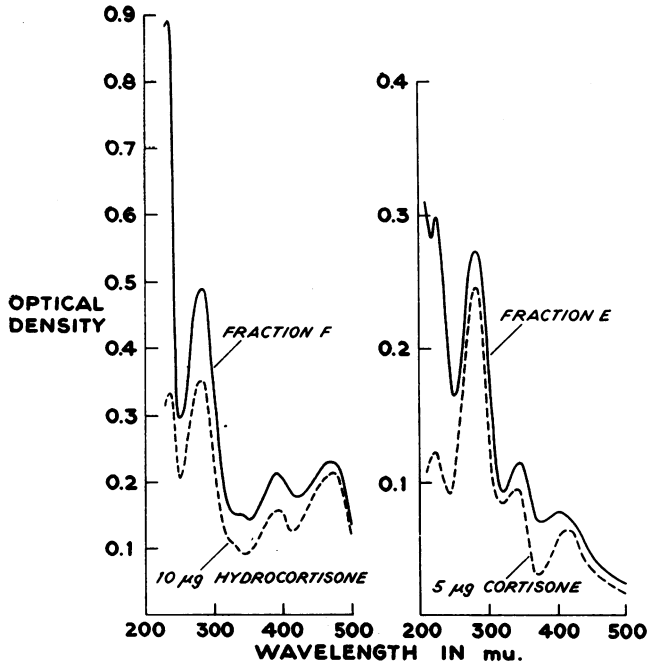

FIG. 3.—Absorption spectra of the sulfuric acid chromogens of salmon F and salmon E fractions.

parallel with the corresponding standards. It appears that the major steroid is hydrocortisone with a small amount of cortisone. Fraction B area was also run in the Bush system with corticosterone as the reference standard. No ultraviolet absorbing area corresponding to corticosterone was observed.

Areas of fraction F, E, and B were eluted from the paper and further analyzed. Fraction F had an Rf similar to the standard hydrocortisone. Aliquots of fraction F were checked by the "blue tetrazolium" reaction and the absorption spectra of the sulfuric acid chromogens. A positive "blue tetrazolium" reaction was obtained indicating an alpha ketol. Absorption spectra compared to that of hydrocortisone are shown in Figure 3.

Fraction E had an Rf similar to cortisone. Aliquots of fraction E were analyzed as described for fraction F. "Blue tetrazolium" reaction was positive, and the

absorption spectra was similar to that of standard cortisone as shown in Figure 3. Analysis of the fraction B area showed no detectable amounts of any of the $C_{21}O_4$ steroids.

Pooled male and female plasma from maturing salmon (Mill Creek), when chromatographed and analyzed, showed the same pattern of corticosteroids as the plasma from spawning female salmon. Similar analysis of spawning steelhead trout plasma gave analogous results.

TABLE 5

SUMMARY OF AVAILABLE DATA ON CONCENTRATION OF PLASMA 17-HYDROXYCORTICOIDS IN SEXUALLY MATURING AND SPAWNING FISHES

Reported by	Kind of Fish	Sex	During Sexual Maturation, μg/100 ml	At Spawning or Post-Spawning, μg/100 ml
Hatey,[14] Fontaine and Hatey[15]	Atlantic salmon	M	52.2	31.7
		F	45.3	30.6
Hane and Robertson	Pacific salmon (*O. tschawytscha*), Sacramento river	M	45.2	31.3
		F	53.4	78.7
Hane and Robertson	Pacific salmon (*O. tschawytscha*), Columbia river grilse	M	89.0	...
		F	...	...
Hane and Robertson	Rainbow trout, migratory (steelhead)	M	...	22.9
		F	...	35.2
Hane and Robertson	Rainbow trout, nonmigratory (hatchery)	M	...	10.0
		F	...	...
Bondy, Upton and Pickford[16]	Carp	M	...	24.4
		F	...	43.8

Discussion.—Very few studies of the adrenal corticosteroids in fishes have been reported. Phillips and Chester Jones[13] identified such steroids in the ray as corticosterone (8 μg/100 ml plasma), in the dogfish as hydrocortisone (2–3 μg/100 ml whole blood), in the cod as hydrocortisone (1 μg/100 ml whole blood), and in the lungfish as hydrocortisone (15 μg/100 ml plasma). No mention was made of the state of sexual development in these fish. The only determinations of 17-OHCS concentrations in salmon were made by Hatey[14] and Fontaine and Hatey.[15] These authors studied the plasma corticoids of young Atlantic salmon (*S. salar*) on their seaward migration and found 19.6 μg/100 ml in the parr and 85.5 μg/100 ml in the smolt. Their determinations on maturing and spawning salmon of the same species are given in Table 5, which summarizes the available data (including our own) on 17-OHCS levels in salmon, trout, and carp accompanying sexual development and spawning.

It will be noted that the corticoid concentrations (heart's blood plasma) of the two types of salmon, Atlantic and Pacific, during sexual maturation are essentially alike. However, at spawning the Atlantic salmon of both sexes showed a decided drop in 17-OHCS; whereas in the Pacific salmon, only the males showed this change. In contrast, the spawning female Pacific salmon exhibited a rise in corticoids to a much higher level. A similar, though less pronounced, difference in 17-OHCS concentration between male and female was found in the steelhead trout. Bondy, Upton, and Pickford[16] observed an analogous phenomenon in post-spawning carp. Histological examination of the adrenal cortical tissue of our spawning salmon and steelhead revealed no apparent difference in the degree of degeneration between males and females.

The progressive increase in the concentration of plasma 17-OHCS in the maturing

female Pacific salmon is not unlike that in the pregnant human female,[17, 18] in whom there is also hypertrophy of the adrenal glands.[19] While the mean levels of plasma 17-hydroxycorticosteroids in the third trimester of pregnancy found by the above authors (24 to 49 μg/100 ml) are lower than those of the sexually mature female salmon, the concentration rises at the time of labor and may reach 80 μg/100 ml[18] which is almost identical with our finding in the spawning salmon. The amounts of corticoids found in cases of Cushing's syndrome vary considerably, but in many such cases plasma levels are approximately the same as those in sexually maturing salmon, i.e., 40–50 μg/100 ml.

The increasing concentrations of 17-OHCS in the blood of the Pacific salmon as they progress from the sea to the spawning grounds parallels in general the inception and degree of adrenal hyperplasia described in an earlier study.[5] By the time the salmon was ready to spawn the amount of adrenal cortical tissue had increased many fold over that present in the immature fish in the sea. The hyperplasia appeared to be equally pronounced in males and females.

The results of this study do not reveal any unequivocal relationship between height of 17-OHCS concentration and survival after spawning among the salmonids, with the possible exception of the nonmigratory rainbow trout. These fish usually survive their first spawning with a corticoid concentration of only 10 μg /100 ml. Steelhead trout and Atlantic salmon both exhibit a considerable post-spawning mortality and have an elevated plasma corticoid content. Yet carp, which are known to spawn repeatedly, have been shown to possess an equally high 17-OHCS concentration.

The significance of the well-established marked increase in the plasma concentration of adrenal corticosteroids lies in their catabolic effects on the body organs and tissues. No information on this subject in fish is available. Forthcoming communications will deal with histological and physiological changes associated with these abnormally high concentrations of 17-hydroxycorticosteroids.

Summary—Plasma 17-hydroxycorticosteroids have been determined in king (Chinook) salmon at successive stages of sexual development from complete immaturity in the sea to full maturity on the spawning grounds. Plasma samples taken at two points on the spawning migration showed a marked rise in 17-OHCS concentration in both males and females of 4 to 5 times the normal value at sea of 11.8 μg /100 ml. At spawning the females showed a further rise to a mean of 79 μg /100 ml, while the concentration in the males fell to 31 μg /100 ml. Chromatographic studies of the plasma of sexually mature salmon revealed the presence of hydrocortisone and cortisone, predominantly the former. Absorption spectra of the sulfuric acid chromogens and Rf values gave further confirmation of the nature of these two corticoids. No other corticosteroids were detected. Concentrations of 17-OHCS in the plasma of spawning anadromous rainbow trout (steelhead) averaged 23 μg /100 ml for the males and 35 μg /100 ml for the females. Chromatographic analysis demonstrated the same corticoids as in the salmon. In sexually mature nonmigratory male rainbow trout (hatchery reared) a concentration of only 10.0 μg /100 ml was present.

The relationship of the plasma corticoids to the degree of adrenal hyperplasia has been discussed.

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[12] Due to the rapidity with which the salmon were caught on the collecting trip to the Bolinos Bay region, the interval between capture and bleeding (followed by autopsy) became progressively longer. In spite of the fact that the fish were held in a large tank supplied with an abundant flow of fresh sea water many of them were listless and some lying on their sides by the time we were able to bleed them. The last fish, bled after 2 hours, was quite immobile. There was a progressive rise of the Porter-Silber chromogens with time after capture from 7.0 (1 fish), 13.2 (mean of 7 fish), 23.8 (mean of 9 fish), to 29.2 (1 fish) μg/100 ml at 2 hr. In the other experiments reported in this paper less than 10 min elapsed between catching and bleeding.

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Addendum: As this paper was going to press our attention was called to a report by Idler, Ronald, and Schmidt on the isolation of cortisone and cortisol from the blood plasma of Pacific salmon (*Oncorhynchus nerka*) which appeared in the March 5, 1959 *J. Am. Chem. Soc.* These workers found 17μg of hydrocortisone (cortisol) and 37 μg of cortisone per 100 ml plasma in salmon just before arrival at the spawning ground. This ratio of the two corticoids is the reverse of that present in the blood of the king salmon *O. tschawytscha.*