Presence of ACTH in Pituitary Gland of Pacific Salmon (*O. keta*). (22080)

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The existence of a relationship between the pituitary gland and interrenal tissue of fish (*Astyanax mexicanus*) has been reported by Rasquin(1). Following injections of ACTH of mammalian origin, the anterior interrenal tissue, which is considered homologous with the mammalian adrenal cortex, responds by hypertrophy, hyperplasia, and increased vascularization. Hatay(2-4), and Fontaine and Hatay(5-7) show that the corresponding tissue in the eel (*Anguilla anguilla* L) is rich in ascorbic acid and that following hypophysectomy, this component is decreased and the interrenal tissue has become atrophic. In contrast, Pickford(8) was unable to detect changes in the cortical tissue of *Fundulus* following hypophysectomy.

It seemed of interest to determine if a corticotropic substance exists in the teleost pituitary, which like mammalian ACTH, can deplete adrenal ascorbic acid in the hypophysectomized rat. Assays of lyophilized pituitary glands* and of certain pituitary fractions of a species of Pacific salmon (*Oncorhynchus keta*), which will be reported, show clearly the presence of ascorbic acid-depleting activity.

Materials and methods. Preparation of extracts. Pituitary glands from freshly caught salmon were preserved in solid carbon dioxide within 3 hours of sacrifice. Following lyophilization, the dried tissue was ground with mortar and pestle. Immediately before use a weighed amount of tissue was extracted in an appropriate volume of N/1000 hydrochloric acid. This preparation was injected without further processing. *Preparation of fractions.* Fraction #1. Lyophilized pituitary tissue was extracted at room temperature for 24 hours in a solution of glacial acetic acid and acetone;† one g of tissue in 16 ml of acid and 2.5 ml acetone. One volume of distilled water was then added. After thorough mixing the suspension was centrifuged, the insolubles being preserved by lyophilization for further study. To the supernatant acetone was added, with stirring, until heavy turbidity was evident. At this point additional acetone was added cautiously until a flocculent precipitate formed, leaving a clear supernatant. The solids were collected by centrifuging, and washed twice with ethyl ether. The washed precipitate was redissolved in water, enough glacial acetic acid being added to assure solution. After warming at 37°C for a few minutes to remove excess ether, the solution was frozen and lyophilized. Yield: approximately 13% of starting weight. Fraction #2. To the supernatant fluid remaining after the precipitation of Fraction #1 was added at least one volume of acetone. If necessary,

TABLE I. Adrenal Ascorbic Acid Depletion in Hypophysectomized Rats following the Intravenous Administration of Various Pituitary Preparations of the Pacific Salmon (*O. keta*).

Preparation used	Dose* (μg)	No. of animals	Adrenal ascorbic acid depletion (mg %)
Lyophilized tissue	25	3	73, 68, 105
	100	3	101, 177, 119
Fraction #1	50	2	222, 188
	100	3	214, 167, 182
Fraction #2	50	3	137, 105, 150
	100	3	149, 156, 70
Fraction #1	10	3	83, 138, 97
	20	2	124, 96
	40	4	176, 143, 195, 162
Fraction #2	12.5	3	37, 79, 62
	25.0	3	72, 64, 84
	50.0	3	159, 134, 177

* Dose based on 100 g of body wt. Left adrenals removed immediately before intravenous administration of test dose. Right adrenals removed 1 hr following test dose.

* Pituitary glands were kindly supplied by Mr. Roger Burrows and his colleagues of the Fish and Wildlife Service, U. S. Department of Interior, Entiat, Wash.

† This procedure is a modification of the method reported by Payne, Raben and Astwood(9) for extraction of hog pituitary glands.

TABLE II. Adrenal Ascorbic Acid Depletion Assays in Hypophysectomized Rats Injected with Pituitary Preparations of the Pacific Salmon *(O. keta)* and with U.S.P. Standard Corticotropin.

No. of animals	Test material	Test dose per 100 g body wt	Mean response, adrenal ascorbic acid depletion	Assay data
8	LSP*	25 μg	110	$S_c = \pm 20$†
8	"	50 "	131	$b_c = 86.7$
8	"	100 "	160	$\lambda_c = .23$
7	USP Std	.5 mU§	103	$R\frac{(USP)}{(LSP)} = 48.9$‡
7	"	1.0 "	134	95% limits 35.7-66.8
7	"	2.0 "	158	
3	Fraction #1	25 μg	109	$S_c = \pm 32$
4	"	50 "	168	$b_c = 143$
3	USP Std	.5 mU	93	$\lambda_c = .22$
3	"	1.0 "	120	$R\frac{(USP)}{(Fr.1)} = 29.4$
3	Fraction #2	25 μg	85	
3	"	50 "	95	$S_c = \pm 24$
3	"	100 "	130	$b_c = 84.3$
3	USP Std	.5 mU	93	$\lambda_c = .29$
3	"	1.0 "	120	$R\frac{(USP)}{(Fr.2)} = 52.8$
3	"	2.0 "	149	

Assays conducted according to the method of Sayers *et al.* Left adrenals removed immediately before injection of test dose. Right adrenals removed 1 hr following test dose.

* LSP = Lyophilized salmon pituitary tissue.

† S = Stand. dev.; b = slope; $\lambda = s/b$; subscript 'c' for combined data.

‡ R = Ratio of potency.

§ mU = Milliunits.

further acetone was added to reduce the turbid suspension to a flocculent precipitate. The solids were collected by centrifuging and prepared for lyophilization as with Fraction #1. Yield: approximately 11% of starting weight. *Assays.* Adrenal ascorbic acid depletion assays were conducted according to the method of Sayers, Sayers, and Woodbury (10). Male Sprague-Dawley rats, 125-150 g body weight, were hypophysectomized 24 hours prior to assay. Test preparations dissolved in N/1000 hydrochloric acid, were injected, after laparotomy, via the vena cava. Preliminary tests were made to determine whether ACTH activity was present. The results of these were used to estimate dose levels in assays against the USP standard corticotropin.

Results. Data obtained in preliminary tests are presented in Table I. It is apparent that, in these preparations from salmon pituitary tissue, a physiological activity is present which is qualitatively the same as that observed in pituitary preparations of mammalian origin. Potency assays against the USP standard corticotropin are summarized in Table II. The results indicate an ACTH content in the lyophilized tissue of about 20 milliunits per mg. Because of difficulties in field conditions under which these glands were collected, freezing in solid carbon dioxide was delayed 2 to 3 hours after removal. This lapse of time may have resulted in loss of physiological activity. Hence it is possible that freshly frozen glands would show a greater ACTH content.

Assay data on Fractions #1 and #2 indicate some concentration of ACTH activity in the former. On a weight basis Fraction #2 appears about as active as the starting material.

Discussion. The findings reported establish the presence of ACTH activity in the teleost pituitary. A recent report by Nelson, O'Connell, and Haines(11) describes the presence of ACTH activity in sardine fish meal. The results described here may account

in part for this finding, if small quantities of pituitary tissue were present in the meal.

The presence in the teleost of a pituitary component capable of producing adrenal cortical stimulation in the mammal suggests that the protein or peptide structure responsible for this activity was of metabolic importance early in the evolutionary development of the species. Whether or not the role of ACTH in the phenomena associated with response to stress in mammals is the same in fish remains to be determined.

Summary. Lyophilized pituitary tissue of the Pacific salmon (*O. keta*) and pituitary fractions, were examined for ACTH activity in hypophysectomized rats. The results indicate the presence of a component in the teleost pituitary gland capable of depleting the stores of adrenal ascorbic acid in the rat. In this respect the preparations are qualitatively identical with corticotropins (ACTH) of mammalian origin.

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Rate of Glycogenesis in the Liver of the Rat After Parenteral Administration of Glucose, Fructose and Invert Sugar.* (22081)

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It is generally considered that fructose forms glycogen in the liver more rapidly than glucose. This assumption is based upon *in vitro* experiments(1) and studies in which the two sugars were given to rats by mouth (2). Since in clinical practice sugars are administered intravenously for supplying calories to patients unable to take food by mouth, it was considered of interest to study the rate of glycogenesis in the liver when glucose, fructose, and invert sugar are given parenterally.

Method. Normal white rats, weighing 200 to 300 g, were fasted for exactly 24 hours, water being allowed *ad lib*. Sugar, in 10% solution, was injected intraperitoneally. A dosage of 2 g per kg of body weight was used. This dosage was selected because it was found by experiment to give a suitable load to the animal and it is below the level at which utilization might be diminished by biological limitations. A carefully measured quantity of sugar solution was injected intraperitoneally into the fasted rat. At different intervals after administration, animals were anaesthetized with nembutal and the whole liver was removed for analysis. Liver glycogen was determined by an anthrone method developed in our laboratory(3). Our procedure was to give sugar solution to 6 rats and the same volume of physiological saline to 2 control animals. Liver glycogen determinations were then carried out upon the group of 8 animals. Groups of animals were sacrificed at approximately hourly intervals for periods

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