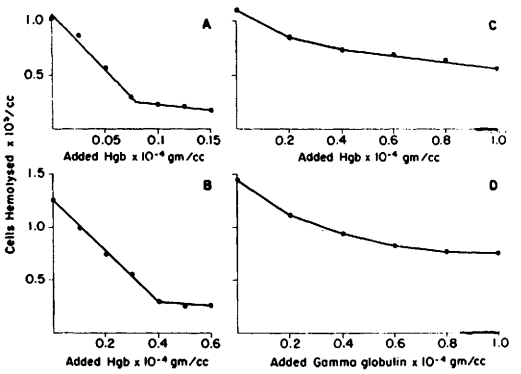

FIG. 1. Complement, hemolysin, and red cells are constant; the resultant hemolysis is plotted against concentrations of interfering protein. A. Complement source: Pooled human serum. Interfering protein: Sheep hemoglobin. B. Complement source: Guinea pig serum. Interfering protein: Sheep hemoglobin. C. Complement source: Pooled human serum. Interfering protein: Pooled human hemoglobin. D. Complement source: Pooled human serum. Interfering protein: Human gamma globulin.

the curves cannot be accurately defined from the data presently available.

The inhibition of complement by gamma globulin agrees with previous reports(7,8,9).

Summary and conclusions. Measurements of relative hemolytic activity of varying mixtures of complement, red cells, and hemolysin with added heterologous protein reveal inhibition or apparent partial complement fixation in the absence of known previous immunization. The added protein in these studies is either hemoglobin or human gamma globulin. This inhibition is absent when the hemoglobin and complement are derived from the same specimen of blood. These data reveal an important possible source of error when quantitative estimates of antibody formation are made using complement fixation tests.

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In vitro Effect of Mammalian Adrenocorticotropin on Secretion of Skate (*Raja erinacea*) Interrenal Tissue.* (27541)

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Although adrenocorticosteroids have been identified in plasma of lower vertebrates(1), the steroidogenic action of adrencorticotropin (ACTH) has not been investigated directly to any extent. *In vitro* stimulation of adrenocortical secretion by mammalian ACTH has been shown for the American bullfrog (*Rana catesbeiana*)(2,3) and suggested for the White Leghorn Cockerel (*Gallus domesticus*)(4). The steroidogenic action of ACTH has also been demonstrated *in vivo* in several avian species(5,6). The present report suggests an *in vitro* steroidogenic effect of mammalian ACTH on skate interrenal tissue. As a by-product, data were also obtained demonstrating differences in skate interrenal

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TABLE I. Body and Interrenal Weights in Male and Female Skate *(Raja erinacea)*.

Sex		No.	Body wt, mg	Interrenal wet wt		
				Unadjusted mean wt		Adjusted mean wt
				mg	mg/100 g body wt	mg
♂		12	748 ± 490*	11.3 ± 5.32*	1.51	11.6
♀	Nonovulating	9	548 ± 214	12.9 ± 5.56	2.36	12.9
	Ovulating	12	630 ± 97	21.5 ± 3.11	3.41	21.5
	Combined	21	595 ± 163	17.8 ± 6.03	3.05	17.8

* Mean ± S.E.

weights related to sex and reproductive activity.

Methods. Common skate† (*Raja erinacea*) weighing 195-1920 g each were captured in Nantucket Sound (July-Sept., 1961), transported in cooled, oxygenated sea water to our laboratory, and maintained at least one week prior to use at 18 ± 2°C in sand-filtered, recirculated sea water‡ (pH 7.9-8.3). Skate were decapitated. Interrenals were removed immediately, trimmed of adherent tissue, blotted on filter paper, and weighed individually on a torsion microbalance. Each gland was halved or quartered, and the pieces distributed equally among 2 or 4 sectors in a Petri dish humidor kept on ice. Pooled interrenal tissue from each sector, usually in excess of 50 mg and representative of each skate, was weighed and transferred to a vessel containing 2 ml ice-cold elasmobranch-bicarbonate-glucose saline (pH 7.3)(8). Vessels were flushed for 10 minutes with 95% O_2-5% CO_2, sealed, and incubated at 18°C in a Dubnoff metabolic incubator. After preincubation for 30 minutes, incubation was carried out for 2 additional hours in fresh medium with ACTH (Wilson oxycellulose purified corticotropin, Lot #10221, 90 U.S.P. units/mg)§ in doses of 1000 MU/100 mg tissue added to one flask of each pair. Upon completion of incubation, the preincubation and incubation fluids were extracted separately with spectroscopic methylene dichloride, duplicate measurements made on unpurified, dried extract residues with blue tetrazolium (BT) and by measurement of peak absorption in the ultraviolet at 240 mμ (UV), and output rates computed as μg corticosterone equivalents/100 mg interrenal (wet wt)/2 hrs incubation as previously described(9).

Results. Individual interrenal and body weights were obtained for male and for nonovulating and ovulating female skate. Criteria for ovulation were the visualization of mature ova or maturing ovarian follicles. Table I shows that average interrenal weights in the common skate, whether expressed as absolute weight or per 100 g body weight, appear greater in ovulating females than in either males or nonovulating females. Since a reasonably strong but not perfect correlation exists between body and interrenal weights of males ($r = 0.776$) and of the combined females (nonovulating and ovulating) ($r = 0.498$), statistical comparison of sex differences and effect of reproductive state was made between interrenal weights adjusted (independent of body weight differences) by covariance correction(10). These values (last column, Table I) are essentially similar to corresponding unadjusted values. Application of the F-test for statistical significance between adjusted mean interrenal weights shows (Table II) that those of males are significantly less than those of either the combined or ovulating females but not significantly different from those of nonovulating females. Interrenal weights of ovulating females are significantly greater than those of nonovulating females.

† Skate were obtained through the cooperation of Mr. Carl Schweidenbach, M.B.L., Woods Hole, Mass.

‡ The aquarium design was furnished by Charles Wheeler, U. S. Fish and Wildlife Service Labs., Woods Hole, Mass. Sea water was obtained at Woods Hole and furnished through the cooperation of Mr. Frederick C. Wilbour, Jr.

§ We are indebted to Dr. C. E. Graham, Wilson Labs., Chicago, Ill., for supplying ACTH.

TABLE II. Statistical Evaluation of Interrenal Weight Sex Differences in Common Skate *(Raja erinacea)*.

Groups compared	Degrees of freedom	F	P
♂ *vs* combined ♀	30	12.61	<.01 >.001
♂ *vs* nonovulating ♀	18	.56	<.50 >.10
♂ *vs* ovulating ♀	21	50.62	<.001
Nonovulating *vs* ovulating ♀	18	22.28	"

Average BT and UV output rates obtained for male and for female interrenal tissue during preincubation and subsequent incubation in absence and presence of ACTH are summarized in Table III. No attempt was made to segregate interrenals from nonovulating and ovulating females because of difficulties in obtaining sufficient quantities of each tissue at any one time. Preincubation BT and UV output rates are approximately of the same magnitude in both sexes and substantially greater than their counterparts during subsequent incubation. Mammalian ACTH stimulated secretion as indicated by substantial increases in both BT and UV output rates in interrenals of both sexes. However, statistical parameters of the significance of the observed ACTH effect and of suggested sex differences in secretory rates were unjustified on the basis of the available number of experiments.

Discussion. Our observations on absolute and relative interrenal weights in both sexes of *Raja erinacea* are similar in magnitude to those of females but are less than those of males of this species reported previously(11). These differences cannot be explained on the basis of existing evidence especially since in both studies the skate were obtained in the same general geographic area and during similar seasons. The earlier study also reported no significant differences between geometric interrenal weights of males and of females unsegregated as to reproductive state. This is similar to our finding with respect to interrenal weight differences between males and nonovulating females. On the other hand, our data do suggest statistically significant inter- and intra-sexual differences in interrenal weights apparently related to reproductive activity. Considerable evidence exists supporting the effects of sex differences and reproductive activity on adrenal weight and secretion in vertebrates. Thus, it is well established that in some eutheria absolute and relative adrenal weight is greater in the female than in the male, and it may be that in the dogfish and torpedo hypertrophic interrenal changes associated with sexual maturity may reflect increased secretion(12). Recently it has been reported that interrenal hyperplastic changes and increased concentrations of plasma cortisol occur in both sexes (cortisol concentration higher in the female) of 2 species of spawning Pacific salmon(13,14).

It is apparent from the present findings that elasmobranch interrenal tissue secretes BT and UV measurable substances and responds to mammalian ACTH *in vitro*. Our findings that output rates during preincubation are substantially greater than incubation rates in absence of ACTH resemble findings obtained for rat adrenals by similar methods(15). Although output rates both in the absence and presence of ACTH are approximately 1/10 those established by similar analytical methods for incubated rat adrenals(9), they compare favorably in mag-

TABLE III. *In vitro* Secretion of Skate *(Raja erinacea)* Interrenal Tissue in Absence and Presence of Mammalian ACTH.

			Output (μg/100 mg/2 hr)							
			BT				UV			
Incubation period	Sex	No.	No ACTH	ACTH	Change μg	Change %	No ACTH	ACTH	Change μg	Change %
Preincubation (½ hr)	♂	5	10.16	—	—	—	17.16	—	—	—
	♀	7	8.48	—	—	—	14.00	—	—	—
Incubation (2 hr)	♂	4	1.28	3.59	2.31	181	1.86	3.00	1.14	62
	♀	4	1.98	4.76	2.78	140	2.31	4.23	2.17	94

nitude with *in vitro* BT output rates reported for American bullfrog adrenals(2). Because of low secretory rates and difficulties in obtaining sufficient quantities of tissue, qualitative analysis of steroids produced *in vitro* by interrenals of *Raja erinacea* was not attempted in this preliminary investigation. However, characteristic reactions with BT and UV provide strong presumptive evidence that the secretory products measured were α ketol-Δ^4-3 ketosteroids. Phillips(7) has shown definitively that cortisol is the predominant plasma corticoid of the female skate (*Raja eglanteria*).

Summary. The effect of mammalian ACTH on *in vitro* secretion of skate (Raja erinacea) interrenals was investigated directly. Interrenal weights also were compared between males and nonovulating and ovulating females. ACTH increased interrenal output of BT and UV measurable substances in both sexes. Preincubation output rates in the absence of ACTH were substantially greater than those during subsequent incubation. A strong but not perfect correlation was observed between body and interrenal weights in both sexes. F-test analysis for statistical significance of differences between means of covariance-corrected interrenal weights showed that those of males were significantly less than those of either combined or ovulating females but not different from those of nonovulating females. Ovulating females had significantly heavier interrenals than nonovulating females.

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Erythropoietic Stimulating Factor (ESF) as a Stimulant of Tumor Growth.* (27542)

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Partial hepatectomy (PH) of rats is known to be followed by regeneration of the liver(1,2), and increased mitotic rate in the cornea. Increased compensatory hypertrophy of the kidneys is also observed after unilateral nephrectomy and PH(3). Evidence that this growth stimulating effect is due to a bloodborne agent is demonstrated by the ability of PH in one member of a parabiotic pair of rats to stimulate mitotic activity in the liver of the intact parabiont(4). Partial hepatectomy also results in a decreased rate of liver regeneration in these animals when compared with nonparabiont, PH controls(5).

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