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by

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ABSTRACT

The effects of estradiol and ovine growth hormone (GH) on liver and plasma protein of hypophysectomized Natrix fasciata (southern banded water snake) were investigated. Liver and plasma protein concentrations were determined colorimetrically and expressed as mg/gm wet weight tissue.

Significant increases in liver protein levels occurred 48 and 72 hours after a single injection of estradiol in hypophysectomized animals. A single injection of GH had a like effect. Estradiol had a greater effect on liver protein levels than did GH. When estradiol and GH were administered together to hypophysectomized animals there was no apparent additive effect of the two hormones in increasing liver protein; the increase in liver protein being essentially like that of estradiol treatment alone.

The two hormones did not promote an increase in plasma protein concentrations of hypophysectomized animals.

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INTRODUCTION

Administration of estrogen in most oviparous or ovo-viviparous vertebrates stimulates vitellinogenesis characterized by an increase in liver weight, an increase in plasma vitellin (phospho-protein), and an increase in liver protein synthesis (Dessauer & Fox, 1959; Hahn, 1967; Wallace & Jared, 1968; Boone, 1968; Jordan, 1969). Additional studies have established that estrogen acts directly on the liver to evoke the synthesis of new RNA molecules which precedes the de novo synthesis of protein (Noteboom & Gorski, 1963; Hahn & Gorbman, 1967; Jordan, 1969).

Although many investigations have focused on the effects of estrogen on the liver in vitellinogenesis, little attention has been given to the possible involvement of the hypophysis. In a recent study in lizards (Dipsosaurus dorsalis and Sceloporus cyanogenys), not only was the hypophysis necessary for the estrogen-induced hepatic and ovarian growth associated with vitellinogenesis, but also a hypophysial factor was needed. This hypophysial factor was believed to be growth hormone (GH) (Callard, personal communication). He proposed that estrogen has a dual action in vitellinogenesis. First, it stimulates the synthesis of new RNA species at the site of the liver for the production of vitellinogenic proteins and, second, it elicits the release of GH necessary for the synthetic

activity of the liver.

Boone (1968) using the isolated perfused liver of Natrix fasciata (southern banded water snake) found that estradiol alone induced vitellinogenesis without the aid of the pituitary. It might be argued, however, that his perfusate, which consisted of 60% whole blood pooled from snakes having intact pituitaries, contained GH or some other hypophysial factor necessary for the vitellinogenic response.

The present study was undertaken to determine the necessity of the pituitary for the estrogen-induced hepatic vitellinogenic response in N. fasciata and to examine the possible interactions of estradiol and GH in this respect. Since estrogen induced an increase in one of the protein fractions of the plasma in Uta stanaburiana (lizard) (Hahn, 1967) and in N. fasciata (Boone, 1968) which was associated with vitellinogenesis, the plasma protein fractions were also investigated in the present study.

METHODS AND MATERIALS

A total of 67 adult female snakes obtained from the Tote-Em-In Zoo, Wilmington, North Carolina in late May and early June of 1970 were used in this investigation (Table 1). They were maintained in screen-covered glass aquaria that contained drinking water and were used within a two-week period. All the animals were gravid, and according to the studies of Dessauer and Fox (1959) on Natrix, should have been in an anestrus condition with very low circulating levels of estrogen. Thus the vitellinogenic response should be obtained with added estrogen.

Twelve snakes served as an initial control group. These animals received a single intraperitoneal injection of 1 ml sesame oil.

A dose of 1.0 mg estradiol benzoate (in 1 ml sesame oil) per 100 gm body weight was administered intraperitoneally to 12 snakes to test the sensitivity of the liver to added estrogen. The dosage was selected on the basis of studies by Boone (1968) and Jordan (1969).

Liver and plasma protein concentrations were determined 48 and 72 hours after injection. The 24 hour experimental period was eliminated on the conclusion from previous studies of Jordan (1969) that no increase in liver proteins was observed 24 hours after estradiol treatment.

Hypophysectomy was performed on the remaining animals using a ventral approach through the roof of the mouth

(Drager, 1949). All operations were performed under Nembutal (Abbott Laboratories) anesthesia and supplemental hypothermia. Eleven snakes served as a second control group. These snakes were untreated and used to determine the effect of hypophysectomy on liver and plasma concentrations of protein. On the tenth day after operation, a single injection of 1.0 mg estradiol benzoate (in 1 ml sesame oil) per 100 gm body weight was administered intraperitoneally to a second group of eleven snakes. Growth hormone (GH, NIH-GH-S9, ovine) was injected intraperitoneally in distilled water, 0.2 iu (200 ug) per 100 gm body weight, to a third group of eleven hypophysectomized snakes.

A fourth group of ten hypophysectomized snakes were examined for the combined effects of GH and estradiol. The dosages of 1.0 mg estradiol benzoate (in 1 ml sesame oil) per 100 gm body weight were administered together intraperitoneally.

Blood samples were collected in heparinized tubes by cardiac puncture from each animal at autopsy and freed of blood cells by centrifugation. The plasma was stored at 4 C until used for plasma protein determination and electrophoresis.

Snakes were killed by severing the neck. From each animal, the entire liver was removed and placed on foil-covered ice. As much connective tissue as possible was removed from the liver. A liver sample of known weight

(approximately 1 gm) was homogenized with three volumes of ice-cold distilled water in a Potter-Elevehjem glass-on-glass homogenizer for five minutes. Liver protein was isolated by the method of Fleck and Munro (1962) and quantitated colorimetrically by a biuret technique (Reinhold, 1953). Total plasma protein concentration was also determined by the biuret technique (Reinhold, 1953).

Plasma protein was electrophoresed using the method of Briere (1964). Samples of approximately 3 lambda were applied to cellulose acetate strips and electrophoresed for 40 minutes at 400 V in a barbital buffer at pH 8.6. Plasma protein fractions were then quantitated using an automatic recording and integrating scanner.

A two-factor factorial experimental analysis of variance for unequal cell frequencies and the Newman-Keuls (q) test were used in the statistical analysis of all data (Winer, 1962). Differences between means were significant at the five per cent level of confidence.

RESULTS

A comparison of intact estradiol-treated snakes with sesame oil controls is given in Table 2. The liver protein concentration of estradiol-treated snakes increased from 126.6 to 155.3 mg/gm wet weight 48 hours after treatment and decreased to 148.0 mg/gm wet weight by 72 hours.

Although these changes were not statistically significant when compared to sesame oil controls, they may have biological meaning in view of similar studies. The plasma protein concentration increased by 37 per cent after estradiol treatment, a significant increase over sesame oil controls.

Plasma protein resolved into five fractions in all groups of animals (figure 1). Table 2 shows that estradiol stimulated significant increases in fractions IV and V.

Table 3 presents a comparison of hypophysectomized animals and sesame oil controls. A significant decrease of about 60 per cent in the liver protein level was observed 13 days after hypophysectomy but there was no change in plasma protein levels or in the plasma fractions.

The effects of treatment of hypophysectomized snakes with estradiol alone, GH alone and with both estradiol and GH are given in Table 4. Hypophysectomized snakes treated with estradiol manifested a two-fold increase in liver protein levels, a significant increase which persisted through 72 hours after treatment. No significant changes

occurred in the plasma proteins. Hypophysectomized animals treated with GH gave a significant increase in liver protein levels which persisted through 72 hours. Plasma proteins did not change. The stimulatory effect of estradiol on liver protein in hypophsectomized snakes was 40 per cent higher than was the effect of GH in hypophysectomized snakes. When estradiol and GH were administered together, liver protein concentration increased significantly by 72 hours when compared to untreated hypophysectomized and GH-treated hypophysectomized animals but did not change in comparison to hypophysectomized animals treated with estradiol alone. Plasma proteins did not change when these comparisons were made.

DISCUSSION

Estradiol stimulated increases in liver protein concentrations in livers of estradiol treated snakes in the present study are in agreement with other investigations. Boone (1968) using the isolated perfused liver of *N. fasciata*, showed that estradiol induced an increase in the uptake of labelled leucine into liver protein. Hahn and Gorbman (1967) in *Uta stanaburiana*, and Jordan (1969) in *N. fasciata* reported that estradiol induced the synthesis of new RNA species which precedes the *de novo* synthesis of protein in estradiol treated livers.

The significant increase (37 per cent) of plasma protein levels in estradiol-treated animals over control values in the present study is in agreement with studies of Dessauer and Fox (1956) who found that the rise in total plasma protein of estrous females averages about 25% in *Thamnophis* (garter snake) and about 15% in *Natrix* over those of nonestrous females. Additional studies by Clark (1967) on fresh-water turtles also demonstrated elevated serum protein levels in response to estradiol administration.

The fact that plasma protein levels were highest 72 hours after estradiol treatment in the present investigation agreed with the findings of Boone (1968). Jordan (1969) postulated that this time corresponds with the declining liver proteins which are being released into the circulation. The fact that plasma protein resolved into five fractions

in all groups of animals in the present study is in agreement with Turner (1967) and Boone (1968). Significant differences between fractions in control and estradiol treated snakes were found only in fractions IV and V. The migratory position of this fraction corresponds to the phospholipoprotein band described for T. sauritus (ribbon snake) by Dessauer and Fox (1959). They proposed that phospholipoprotein is involved in vitellinogenesis, for its sharp decrease following ovulation is correlated when circulating levels of estrogen were once again low in the serum. Hahn (1967) using U. stansburiana reported that estradiol induced a protein fraction which comprised 60-65 per cent of the total plasma protein and is identical in electrophoretic mobility to the plasma vitellin fraction found in females during vitellinogenesis. The protein increase in fraction IV in the present study is similar to the response observed in the phospholipoprotein band in T. sauritus and the plasma vitellin band in U. stansburiana.

Fraction V is believed to be the gamma globulins by the comparison of its slow rate of mobility with that of mammalian plasma (Turner, 1967). No meaningful conclusions can be drawn on the significant increases of this plasma protein fraction in this study.

The effects of hypophysectomy on liver protein in reptiles has received little attention. The marked decrease (60 per cent) of liver proteins in the hypophysectomized

snakes in the present study is as expected, however, when comparison is made to the general protein-depleting effects of hypophysectomy in mammals (Russell, 1957).

The estrogen-induced hepatic protein synthesis (two-fold increase) in hypophysectomized snakes presented in this study is at variance with the findings of Callard (personal communication) who found that an intact pituitary gland is necessary for the estrogen-induced vitellinogenic response of the liver in the lizards Dipsosaurus and Sceloporus. Callard's results suggested that pituitary growth hormone is necessary for the estrogen response. In the present study however, estrogen alone and ovine GH alone were effective in increasing liver protein in hypophysectomized snakes. Estrogen, however, was considerably more effective than GH in this respect. When administered together there was no additive effect, the effect being essentially like that of estrogen administered alone. Thus, in the present investigation an intact pituitary and added ovine growth hormone were not necessary for estrogen-induced hepatic protein synthesis in N. fasciata. This finding substantiates the work of Boone (1968) who found that estradiol alone is capable of stimulating protein synthesis of the isolated liver of N. fasciata.

SUMMARY

1. Plasma protein and plasma fractions IV and V concentrations were significantly higher in estradiol treated intact snakes when compared to sesame oil controls.
2. Liver protein levels were significantly lower in hypophysectomized animals.
3. Liver protein levels in hypophysectomized snakes were significantly higher after estradiol treatment.
4. Liver protein levels in hypophysectomized snakes were significantly higher after growth hormone treatment.
5. The stimulatory effect of estradiol on liver protein synthesis in hypophysectomized snake was 40 per cent greater than was the effect of GH on hypophysectomized snake.
6. When estradiol and GH were administered together to hypophysectomized snakes, liver protein increased significantly over hypophysectomized controls.
7. The combined effect of the two hormones on liver protein synthesis in hypophysectomized animals was essentially like that of estradiol alone.

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TABLE 1

Body Weight and Length of *N. fasciata*

WEIGHT	SNOUT/VENT LENGTH
Sesame Oil Controls	
470.0 gm	772 mm
371.7	692
976	564
144.5	610
419.5	810
120.5	590
125.5	555
120.5	545
185.7	660
542.4	825
375.7	770
269.5	663
Intact-Estradiol treated	
293.5	762
362.6	745
344.6	751
329.0	700
303.6	690
311.0	720
123.0	677
696.0	510
339.0	730
504.2	760
360.0	746
380.0	833
Hypophysectomized	
314.5	710
298.8	715
541.5	818
470.0	872
345.6	786
527.0	798
226.0	660

Table 1 continued

397.8 gm	788 mm
177.0	643
449.5	815
579.4	880
138.6	618

Hypophysectomized-GH Treated

648.0	860
507.5	830
571.2	887
432.5	836
478.2	752
415.4	766
237.7	680
346.0	760
400.5	815
254.5	717
225.5	682

Hypophysectomized-Estradiol Treated

257.0	672
404.4	763
305.0	727
219.0	720
386.0	755
433.5	715
383.2	782
426.0	760
191.3	620
250.0	630
240.5	668
259.4	654

Hypophysectomized-Estradiol and GH Treated

195.3	648
128.0	604
154.2	648
162.0	607
107.5	518
426.3	815
168.4	590
159.6	610
273.0	654
183.3	625

TABLE 2

The Effect of Estradiol on Liver and Plasma Protein Concentrations

	Liver [a]				Plasma [b]				Plasma Fractions [b]							
	48 hours				72 hours								IV			
Comparison	N	$\bar{X}$	SD	q	N	$\bar{X}$	SD	q	N	$\bar{X}$	SD	q	N	$\bar{X}$	SD	q
Sesame oil-controls	6	126.6	6.09	28.70	6	136.6	22.74	11.40	12	5.65	0.75	2.10*	12	2.39	0.56	0.68*
Estradiol	6	155.3	21.70		6	148.0	11.91		12	7.75	1.11		12	3.07	0.84	

	V			
Comparison	N	$\bar{X}$	SD	q
Sesame oil-controls	12	1.51	0.61	0.76*
Estradiol	12	2.27	0.45	

[a] mg/gm wet weight tissue

[b] gm per cent

* Significant at .05 level of confidence

TABLE 3

The Effect of Hypophysectomy on Liver and Plasma Protein Concentrations

	Liver [a]				Plasma [b]				Plasma Fractions [b]							
	48 hours				72 hours								IV			
Comparison	N	$\bar{X}$	SD	q	N	$\bar{X}$	SD	q	N	$\bar{X}$	SD	q	N	$\bar{X}$	SD	q
Sesame oil-controls	6	126.6	6.09	20.00	6	136.6	22.74	51.00*	12	5.65	0.75	0.11	12	2.39	0.56	0.11
Hypophysectomy	6	106.6	29.69		5	85.6	25.10		11	5.76	1.48		11	2.28	0.87	

	V			
Comparison	N	$\bar{X}$	SD	q
Sesame oil-controls	12	1.51	0.61	0.06
Hypophysectomy	11	1.57	0.61	

[a] mg/gm wet weight tissue

[b] gm per cent

* Significant at .05 level of confidence

TABLE 4

The Effect of Estradiol and GH on Liver and Plasma Proteins of Hypophysectomized Snakes

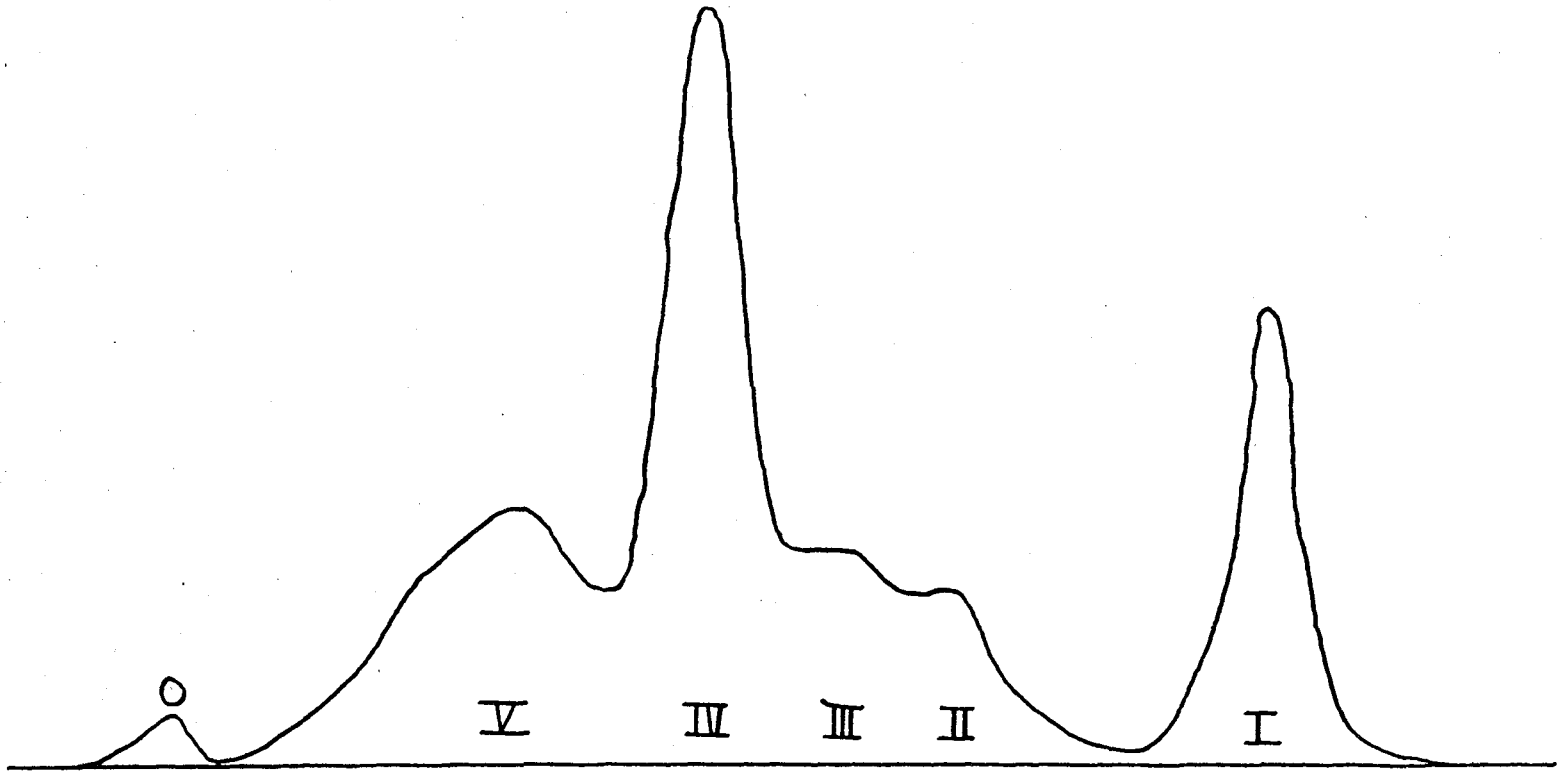
	Liver [a]						Plasma [b]				Plasma Fractions [b]							
	48 hours				72 hours						IV				V			
Comparison	N	$\bar{X}$	SD	q	N	$\bar{X}$	SD	q	N	$\bar{X}$	SD	q	N	$\bar{X}$	SD	q	N	$\bar{X}$	SD	q
Hypox[1] to Hypox & E[2]	6	106.6	29.69	40.60*	5	85.6	25.10	95.50*	11	5.76	1.48	0.43	11	2.28	0.87	0.00	11	1.57	0.61	0.26
	5	147.2	8.15		6	181.1	32.95		11	6.19	1.11		11	2.28	0.79		11	1.83	0.52	
Hypox to Hypox & GH	6	106.6	29.69	46.00*	5	85.6	25.10	42.40*	11	5.76	1.48	0.83	11	2.28	0.87	0.26	11	1.57	0.61	C.32
	6	152.6	28.77		5	128.0	16.97		11	6.56	0.99		11	2.54	0.49		11	1.89	0.51	
Hypox & E to Hypox & GH	5	147.2	8.15	5.40	6	181.1	32.95	53.10*	11	6.19	1.11	0.40	11	2.28	0.79	0.26	11	1.83	0.52	0.06
	6	152.6	28.77		5	128.0	16.97		11	6.56	0.99		11	2.54	0.49		11	1.89	0.51	
Hypox to Hypox & GH & E	6	106.6	29.69	35.80*	5	85.6	25.10	178.4*	11	5.76	1.48	0.06	11	2.28	0.87	0.08	11	1.57	0.61	0.28
	5	142.4	10.61		5	178.4	26.74		10	5.70	1.00		10	2.36	0.53		10	.129	0.67	
Hypox & GH to Hypox & GH & E	6	152.6	28.77	10.20	5	128.0	16.97	50.40*	11	6.58	0.99	0.89	11	2.54	0.49	0.18	11	1.89	0.51	0.60*
	5	142.4	10.61		5	178.4	26.74		10	5.70	1.00		10	2.36	0.53		10	1.29	0.67	
Hypox & E to Hypox & GH & E	5	147.2	8.15	4.80	6	181.1	32.95	2.70	11	6.19	1.11	0.49	11	2.28	0.79	0.08	11	1.83	0.52	0.54*
	5	142.4	10.61		5	178.4	26.74		10	5.70	1.00		10	2.36	0.53		10	1.29	0.67	

a mg/gm wet weight tissue
b gm per cent
1 hypophysectomy
2 estradiol

* Significant at .05 level of confidence

FIGURE 1

Electrophoretogram of the Plasma Proteins of Natrix fasciata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VITA

Terry Chong Der was born on October 24, 1944 in Canton, China. He entered the United States as a naturalized citizen in 1952 and resided in Chicago, Illinois where he completed his primary education in the Chicago city schools and was graduated from Tilden Technical High School in Feburary, 1962. He attended the University of Illinois and later transferred to the University of Missouri, Saint Louis and received a B.A. Degree in Biology in June, 1968. He began graduate study at the University of Richmond in September, 1968. While at the University he was initiated into the Beta Beta Beta Honorary Biological Society. He completed his requirements for the Master of Science Degree in Biology in August, 1970. He will begin work in a biological research field in September, 1970.