

# The Relative Effectiveness of Estrone, Estradiol-17 $\beta$ , and Estrinol in Sex Reversal in the Red-Eared Slider (*Trachemys scripta*), a Turtle with Temperature-Dependent Sex Determination

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In many turtles the temperature during the middle of incubation determines the gonadal sex of the hatchling. Sex steroid hormones have been implicated in temperature-dependent sex determination in the red-eared slider turtle, *Trachemys scripta*; nonaromatizable androgens are involved in male sex determination and estrogens and aromatizable androgens in female sex determination. Administration of exogenous estradiol-17 $\beta$  to eggs incubating at a temperature that normally produces only males can overcome the effect of temperature and result in all offspring being female. Further, estradiol-17 $\beta$  and incubation temperature synergize to produce a greater feminizing effect at intermediate incubation temperatures that produce mixed sex ratios. This study demonstrates that, in the red-eared slider, there is a complex interaction between incubation temperature, different estrogens, and the dosage effect of each hormone. There are changes in potency of different estrogens with incubation temperature such that estrinol is more potent than estrone and estradiol-17 $\beta$  at 26° (an all-male producing incubation temperature), estrone and estrinol are equipotent to each other and more potent than estradiol-17 $\beta$  at 28.8° (an incubation temperature that produced a male-biased sex ratio), and estradiol-17 $\beta$  is more potent than estrone and estrinol at 29° (an incubation temperature that produced equal numbers of males and females). These changes may be due to differences in synergism between the hormones and incubation temperature. Es-

triol treatment also resulted in cranially hypertrophied oviducts at all incubation temperatures in a dose-dependent manner, whereas animals treated with estradiol-17 $\beta$  and estrone had normal oviducts. These results support the hypothesis that estrogens are involved in the final common pathway of female sex determination in this species. © 1996 Academic Press, Inc.

In many reptiles gonadal sex is determined by the temperature of the incubating egg, a process known as temperature-dependent sex determination (TSD). In the red-eared slider turtle (*Trachemys scripta*), incubation of eggs at relatively low temperatures (e.g., 20–28.6°) results in only male hatchlings, whereas relatively high temperatures (e.g., 29.6–35°) results in only female hatchlings; when eggs are incubated at temperatures intermediate to these, varying sex ratios are produced (Crews *et al.*, 1994). Sex steroid hormones appear to be the physiological equivalent of incubation temperature and both male- and female-producing incubation temperatures and exogenous steroids exert their effects during the mid-trimester of development (Crews *et al.*, 1996; Wibbels *et al.*, 1991a). Estrogens and aromatizable androgens induce female sex determination, whereas nonaromatizable androgens induce male sex determination (Bull *et al.*, 1988; Crews *et al.*, 1991, 1994, 1995, 1996; Wibbels and Crews, 1994, 1995; Wibbels *et al.*, 1991b) (Fig. 1).



regressed but visible, normal (as in a typical female hatchling), or hypertrophied; the latter instance was characterized by substantial development and convolutions at the cranial end. The cloacal wall was cut and the development of the phallus noted. Normally, in the female hatchling the cloaca is without pigment and a phallic tubercle is present, with the shaft posterior to the cloacal opening smooth and unmodified. In a hatchling male the tissue surrounding the phallus is pigmented and the shaft has the beginnings of an elementary modification on the distal end. In those instances where the individual had both oviducts and a phallus, the gonads were removed for histological examination. In addition, the gonads of three to five individuals from each experimental group was processed for histological examination of the gonad to confirm sex assignment.

Independent variables in the model were incubation temperature, hormone treatment (estrone, estradiol-17 $\beta$ , or estriol), and dosage of hormone. Sex ratios were tabulated as nominal dependent variables and analyzed with logistic polynomial regression for each hormone at each incubation temperature; a backwards stepwise procedure was used to reduce the models to include only significant regression coefficients (Chatterjee and Price, 1977; Sokal and Rohlf, 1981). Nonsignificant terms (i.e., regression coefficients) were removed at a  $P > 0.1$  to arrive at the final models. Regression coefficients were compared among hormones (within a temperature) and among incubation temperatures (within a hormone treatment) to determine the relative effectiveness of the different hormones and the synergism between temperature and hormones, as described in Crews *et al.* (1995). Synergism between incubation temperature and hormone treatment is present if  $b_1$ 's at intermediate temperatures (i.e., 28.8, 29, and 29.2°) are significantly larger than  $b_1$  at the baseline temperature (26°). In other words, if  $b_1$  is larger at intermediate temperatures than at the baseline temperature, a given dosage of hormone produces a greater effect at intermediate temperature than at baseline temperature.

The logistic regression was utilized for analysis of the frequency of hypertrophied oviducts in estriol-treated turtles. As stated previously, oviducts were classified as normal or hypertrophied and the numbers in each class tabulated. Independent variables were incubation temperature and dosage.

## RESULTS

There were no histological differences detected between the ovaries from hormone-treated hatchlings versus temperature-induced male and female control hatchlings; hermaphroditic (=ovotestes) gonads were not observed even in individuals having both oviducts and a developed phallus.

Incubation temperature significantly affected sex ratios; increasing numbers of females were produced with increasing temperature (the  $b_0$ 's increase at each temperature, Tables 1–3; Fig. 2). All three hormones had significant dosage effects at the lower temperatures; increasing the dose increased the number of females produced at each temperature ( $b_1$ 's all significantly greater than 0). No dosage effect could be calculated for any hormone at 29.2° or for estradiol-17 $\beta$  at 29° because only females were produced at the lowest dosage.

At 26° there was little difference between estrone and estradiol-17 $\beta$  ( $b_1$ 's not significantly different, see Tables 1 and 2). Estradiol-17 $\beta$ , though, was less effective than estrone at the highest dosage (i.e., a significant negative  $b_2$  for estradiol-17 $\beta$ , see Tables 1 and 2). In contrast, estriol was much more potent than E1 and E2 at 26° ( $b_1$  significantly greater than for estrone or estradiol-17 $\beta$ , see Tables 1–3). At 28.8°, there was no difference between the effectiveness of estrone and estriol ( $b_1$ 's not significantly different), although both of these hormones were more effective than estradiol-17 $\beta$  ( $b_1$ 's significantly greater than estradiol-17 $\beta$   $b_1$ ). Comparisons of potency at 29° were only done among hormones at a dosage of 0.01  $\mu\text{g}$  because there was little sex ratio variation above this dosage. There was significant heterogeneity among hormone treatment groups (likelihood ratio  $\chi^2 = 8.4$ ,  $P = 0.015$ ). There was no detectable difference between 0.01  $\mu\text{g}$  estrone and estriol treatments at 29° (LR  $\chi^2 = 0.707$ ,  $P = 0.40$ ). At this dosage and temperature, estradiol-17 $\beta$  produced more females than either estrone (LR  $\chi^2 = 5.06$ ,  $P = 0.025$ ) or estriol (LR  $\chi^2 = 8.34$ ,  $P = 0.0039$ ). At 29.2°, all three hormones produced only female hatchlings at the 0.05  $\mu\text{g}$  dosage and at the 0.01  $\mu\text{g}$  dosage, E1 and E3 produced all females and E2 produced 25/27 females; this effectively prevented calculation of a dosage effect and comparison of the relative effectiveness of these hormones at this temperature.

Synergism between estrone and incubation temperature was evident. A given dosage of estrone had a

TABLE 1  
Separate Regression Results (in Columns) for the Effect of Increasing Estrone Concentrations on the Hatchling Sex Ratio in the Red-Eared Slider Turtle (*Trachemys scripta*) at Three Incubation Temperatures

Regression coefficient	Incubation temperature		
	26°	28.8°	29°
$b_0$	$-4.14 \pm 0.63^a$ ( $\chi^2 = 42.7$ , $P < 0.0001$ ) [ $I = -5.22$ , $u = -3.06$ ]	$-1.28 \pm .51^{*b}$ ( $\chi^2 = 6.4$ , $P = 0.0112$ ) [ $I = -2.20$ , $u = -0.36$ ]	$0.21 \pm 0.25^c$ ( $\chi^2 = 0.75$ , $P = 0.39$ ) [ $I = -0.22$ , $u = 0.64$ ]
$b_1$	$9.8 \pm 1.4^a$ ( $\chi^2 = 49.7$ , $P < 0.0001$ ) [ $I = 7.4$ , $u = 12.2$ ]	$243 \pm 69^{*b}$ ( $\chi^2 = 12.54$ , $P = 0.0004$ ) [ $I = 119$ , $u = 367$ ]	$61.4 \pm 18.5^c$ ( $\chi^2 = 11.0$ , $P = 0.0009$ ) [ $I = 29.7$ , $u = 93.1$ ]

Note. Regression coefficients  $\pm 1$  standard error with Chi-square and probability values in parentheses; there is 1 degree of freedom for each regression coefficient. Regression coefficients with different superscripted letters in bold are significantly different at a level of  $\alpha = 0.05$  for different incubation temperatures. The coefficients are significantly different if their comparison limits in brackets do not overlap. \*These regression coefficients were calculated after removal of data at a dosage of 0.1  $\mu\text{g}$  because the coefficients were unstable when this data set was included. Parameter estimates did not change significantly when these data were removed.

greater feminizing effect at 28.8 and 29.0° than at 26° (i.e.,  $b_1$ 's were significantly larger at 28.8 and 29° than at 26°; Table 1). Estradiol and temperature also had a synergistic effect (i.e.,  $b_1$  at 28.8 was significantly larger than  $b_1$  at 26°, Table 2). In contrast, estradiol and temperature had no detectable synergistic effect (i.e.,  $b_1$ 's not significantly greater at higher temperatures than at the

baseline temperature; Table 3). Although there was an increase from 26 to 28.8° in the percentages of males produced at the lowest dosage of estradiol, the increase in the  $b_1$  regression coefficient was not significant. Second and higher order polynomials were significantly different than 0 for some of the analyses. Third or higher order polynomials are harder to interpret and have no clear biological meaning.

TABLE 2  
Separate Regression Results (in Columns) for the Effect of Increasing Estradiol-17 $\beta$  Concentrations on the Hatchling Sex Ratio in the Red-Eared Slider Turtle (*Trachemys scripta*) at Three Incubation Temperatures.

Regression coefficient	Incubation temperature		
	26°	28.8°	29°
$b_0$	$-4.61 \pm 0.82^a$ ( $\chi^2 = 31.5$ , $P < 0.0001$ ) [ $I = -6.01$ , $u = -3.21$ ]	$-0.55 \pm .33^{*b}$ ( $\chi^2 = 2.86$ , $P = 0.09$ ) [ $I = -1.14$ , $u = 0.04$ ]	n/a
$b_1$	$11.3 \pm 2.8^a$ ( $\chi^2 = 15.7$ , $P = 0.0001$ ) [ $I = 6.5$ , $u = 16.1$ ]	$44.3 \pm 13.3^{*b}$ ( $\chi^2 = 11.05$ , $P = 0.0009$ ) [ $I = 20.4$ , $u = 68.2$ ]	n/a
$b_2$	$-6.7 \pm 2.2$ ( $\chi^2 = 9.1$ , $P = 0.0026$ )	n.s.	n/a

Note. Regression coefficients  $\pm 1$  standard error with Chi-square and probability values in parentheses; there is 1 degree of freedom for each regression coefficient. Regression coefficients with different superscripted letters in bold are significantly different at a level of  $\alpha = 0.05$  for different incubation temperatures. The coefficients are significantly different if their comparison limits in brackets do not overlap. \*These regression coefficients were calculated after removal of data at a dosage of 0.1  $\mu\text{g}$  because the coefficients were unstable when this data set was included. Parameter estimates did not change significantly when this data was removed.

The effect of estradiol on development of the oviducts was pronounced (Figs. 3 and 4). Increased dosage of estradiol increased the number of turtles with hypertrophied oviducts (dosage coefficient =  $364 \pm 130$ , LR  $\chi^2 = 7.88$ ,  $P = 0.005$ ). Importantly, incubation temperature (temperature coefficient =  $5.5 \pm 2.2$ , LR  $\chi^2 = 6.09$ ,  $P = 0.0136$ ) and incubation temperature by dosage interaction (dosage by temperature interaction coefficient =  $-12.4 \pm 4.5$ , LR  $\chi^2 = 7.72$ ,  $P = 0.0055$ ) also had significant effects on the frequency of hypertrophied oviducts. This interaction was due to a decrease in potency of estradiol with increasing incubation temperature (i.e., negative interaction regression coefficient). There was also a small negative second order coefficient for the dosage effect (dosage X dosage coefficient =  $-0.39 \pm 0.15$ , LR  $\chi^2 = 6.93$ ,  $P = 0.0085$ ). These results are opposite to the synergism of the other estrogens with incubation temperature in feminizing gonadal sex.

## DISCUSSION

In many turtles, the temperature of the incubating egg determines the sex of the hatchling. The mechanism

TABLE 3  
Separate Regression Results (in Columns) for the Effect of Increasing Estriol Concentrations on the Hatchling Sex Ratio in the Red-Eared Slider Turtle (*Trachemys scripta*) at Three Incubation Temperatures

Regression coefficient	Incubation temperature		
	26°	28.8°	29°
$b_0$	$-4.87 \pm 1.12^a$ ( $\chi^2 = 19.0, P < 0.0001$ ) [ $I = -6.79, u = -2.95$ ]	$-1.28 \pm .50^b$ ( $\chi^2 = 6.4, P = 0.0111$ ) [ $I = -2.14, u = -0.42$ ]	$-0.05 \pm 0.27^c$ ( $\chi^2 = 0.04, P = 0.84$ ) [ $I = -0.19, u = 0.73$ ]
$b_1$	$151 \pm 43^a$ ( $\chi^2 = 12.5, P = 0.0004$ ) [ $I = 77, u = 225$ ]	$238 \pm 72^a$ ( $\chi^2 = 10.9, P = 0.0009$ ) [ $I = 115, u = 361$ ]	$126 \pm 47^a$ ( $\chi^2 = 7.2, P = 0.0072$ ) [ $I = 46, u = 206$ ]
$b_2$	$-886 \pm 436$ ( $\chi^2 = 4.1, P = 0.0421$ )	n.s.	n.s.
$b_3$	$1238 \pm 716$ ( $\chi^2 = 2.99, P = 0.0840$ )	n.s.	n.s.

Note. Regression coefficients  $\pm 1$  standard error with Chi-square and probability values in parentheses; there is 1 degree of freedom for each regression coefficient. Regression coefficients with different superscripted letters in bold are significantly different at a level of  $\alpha = 0.05$  for different incubation temperatures. The coefficients are significantly different if their comparison limits in brackets do not overlap.

of action of temperature is not well understood, although steroid hormones appear to be involved. There is general agreement that in female sex determination, estrogen formation is the physiological equivalent of incubation temperature. This interpretation is supported by the following observations in our studies with the red-eared slider: (i) Exogenous estrogens can override the effects of a male-producing incubation temperature (this study; Crews *et al.*, 1991; see also Dorizzi *et al.*, 1991; Gutzke and Bull, 1986; Pieau, 1974). (ii) The sex-reversing effect of exogenous estradiol-17 $\beta$  has a critical period that corresponds to the temperature-sensitive window, which extends from Stage 15 through 20 (Gutzke and Chymiy, 1988; Wibbels *et al.*, 1991a,b). (iii) Steroid-induced feminization is mediated via an estrogen-specific receptor (Wibbels and Crews, 1992; see also Crews *et al.*, 1989). (iv) Estrogen is concentrated primarily in the liver and the adrenal-kidney-gonad area of all embryonic stages (Gahr *et al.*, 1992). (v) Administration of the aromatizable androgens testosterone or androstenedione to eggs incubating at a male-producing temperature feminize embryos, presumably through their metabolic conversion to estrogens (Crews and Bergeron, 1994; Crews *et al.*, 1995; Wibbels and Crews, 1992, 1995). (vi) Application of aromatase inhibitor to eggs incubating at a female-producing temperature results in male hatchlings (Crews and Bergeron, 1994; Wibbels and Crews, 1994; see also Dorizzi *et al.*, 1994; Rhen and Lang, 1994). Administration of both testosterone and aromatase inhibitor at a

male-producing incubation temperature blocks the testosterone-induced feminization and all offspring are male. Similarly, administration of testosterone and aromatase inhibitor at a female-producing incubation temperature results in all male offspring. (vii) There is a synergism between exogenous estradiol-17 $\beta$  and estrone and incubation temperature (this study; Wibbels *et al.*, 1991b). (viii) The morphological changes occurring in response to exogenous estrogen are indistinguishable from the changes induced by a shift from a male-producing to a female-producing incubation temperature during the temperature-sensitive window (Wibbels *et al.*, 1993). Taken together, these data are consistent with the hypothesis that temperature and estrogen work in the same developmental pathway for female sex determination.

This raises the question of whether the endocrine environment actually differs with incubation temperature. Contradictory information exists. In the pond turtle (*Emys orbicularis*), Pieau *et al.* (1982) report higher levels of androstenedione and dihydrotestosterone in embryonic testes compared to ovaries. Incubation of gonadal explants from embryos with radioactivity-labeled androstenedione yields little estrogen, but incubation with testosterone yields both estrone and estradiol-17 $\beta$  (Desvages and Pieau, 1991). This suggests that in this species the preferred metabolic pathway is from testosterone to estradiol-17 $\beta$ . Radioimmunoassay (RIA) reveals that during the temperature-sensitive period, gonads from embryos at a female-producing incubation

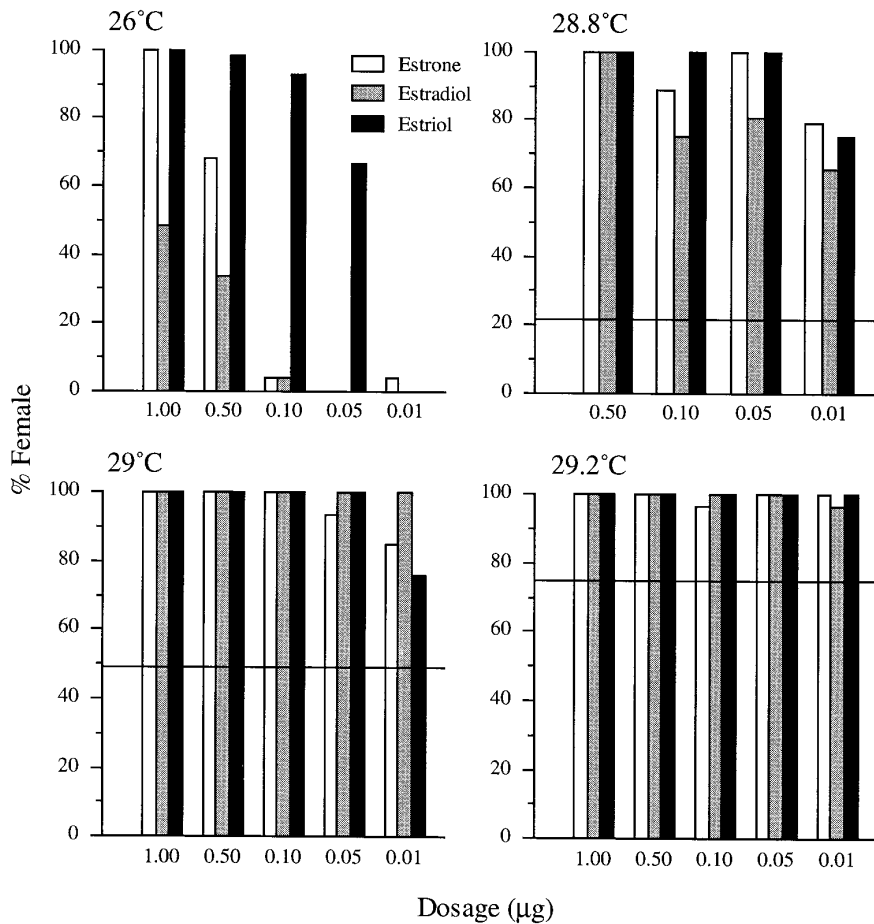


FIG. 2. Effect of different dosages of exogenous estrone, estradiol-17 $\beta$ , and estriol on sex determination in the red-eared slider turtle (*Trachemys scripta*). Eggs were incubated at four different temperatures: 26.0° (all-male), 28.8° (male-biased), 29.0° (1:1 sex ratio), and 29.2° (female-biased). Shown is the percentage of female hatchlings produced at each incubation temperature. Mean percentage of female hatchlings from eggs treated with alcohol alone (control) is represented by the horizontal bar.

temperature have higher estrogen content than gonads at a male-producing temperature (Dorizzi *et al.*, 1991). Further, aromatase activity is very low in undifferentiated gonads; it remains low in embryos at male-producing temperature but increases exponentially in embryos at a female-producing temperature (Pieau *et al.*, 1994; see also Smith and Joss, 1994). Shifting eggs from a male- to a female-producing incubation temperature results in an increase in aromatase activity, whereas the opposite manipulation decreases aromatase activity. These changes do not appear to be due to temperature modulation of aromatase activity, but are believed to result from temperature-induced increases in expression of the aromatase gene.

In contrast, White and Thomas (1992) failed to find detectable levels of progesterone, testosterone, or estra-

diol-17 $\beta$  in the media or in the adrenal-kidney-gonadal tissue from embryonic red-eared slider turtles using highly specific and sensitive RIA. Incubation of embryonic adrenal-kidney-gonadal complexes with radiolabeled pregnenolone indicated only weak precursor conversion with no obvious patterns of sexually dimorphic steroid secretion until after the temperature-sensitive window. It is unlikely that the failure of White and Thomas to detect the presence in the tissue content of key steroid hormones at sex-specific temperatures prior to gonadal differentiation, much less sex differences in steroid hormones, was due to the lack of sensitivity in their technique. Indeed, repeated efforts by this laboratory and by F. George and J. Wilson of the Southwestern Medical Center have failed to detect significant levels of testosterone, estradiol-17 $\beta$ , or dihy-

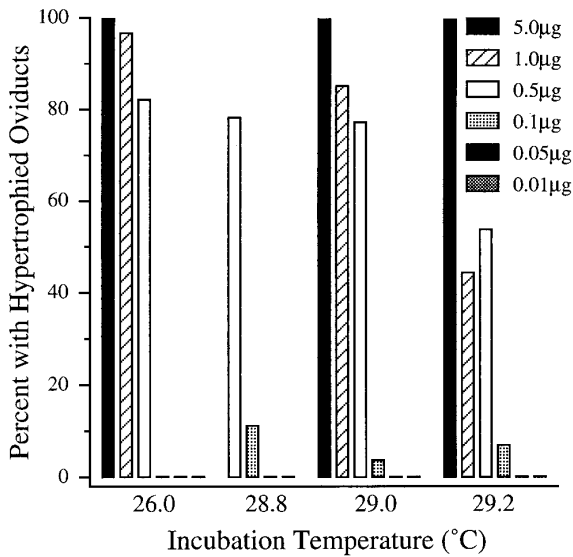


FIG. 3. Effect of varying dosages of estradiol administered at different incubation temperatures on hypertrophy of the oviduct in the red-eared slider turtle (*Trachemys scripta*). The two highest dosages (5.0 and 1.0  $\mu\text{g}$ ) of estradiol were not administered to eggs incubating at 28.8°.

drotestosterone in embryonic gonads or adrenal-kidney-gonadal complexes even though these steroids are readily detected in the serum of adult male and female red-eared sliders. This is puzzling because similar problems have not been encountered with gonads from the European pond turtle or mammalian embryos. It is possible that the red-eared slider turtles differs from the European pond turtle in this as well as other aspects. Several other possible explanations would include: (i) the critical steroids in the red-eared slider embryo are metabolites that do not crossreact in our RIA, (ii) during the temperature-sensitive window this tissue primarily contains kidney and adrenal and the steroid hormone-containing cells may represent only a small fraction of the total, (iii) the poor recoveries obtained from tissue samples (compared to the circulation) indicate that there may be some species-specific tissue factor (glycophospholipid, nonsaponifiable lipid, etc.) that interferes with the extraction and/or chromatography of steroids, (iv) there is some as yet unidentified compound related to steroid hormones that is responsible for sex determination. This latter possibility appears to be the case in *Xenopus laevis* (Hayes *et al.*, 1995).

Androstenedione is a precursor for both estrone and estradiol-17 $\beta$  via aromatase (see Fig. 1). The rate of the reverse reaction from estradiol-17 $\beta$  to estradiol is slower

(Feder, 1981). In mammals estrone and estradiol bind and occupy the same sites of the estrogen receptor as estradiol-17 $\beta$ , but they produce only a weak uterine growth response (Wotiz *et al.*, 1968); indeed, estradiol appears to have antagonistic properties (Lippman *et al.*, 1977). Additionally, in the bovine system cooperative binding of the estrogens to the estrogen receptor is differential; both estrone and estradiol are not cooperatively bound as is estradiol-17 $\beta$  and have a lower capacity than estradiol-17 $\beta$  to induce a positive cooperative interaction with the estrogen receptor (Sasson and Notides, 1983a).

Incubation temperature can synergize with exogenous hormone treatment; eggs incubated at a threshold temperature are more sensitive to estradiol-17 $\beta$  than eggs incubated at an all-male producing temperature (Wibbels *et al.*, 1991b). The present study extends this synergism to estrone, but not estradiol. Thus, the degree of synergism between estrogen and incubation temperature varied as a function of the specific estrogen utilized. When comparing the effects of estrone, estradiol-

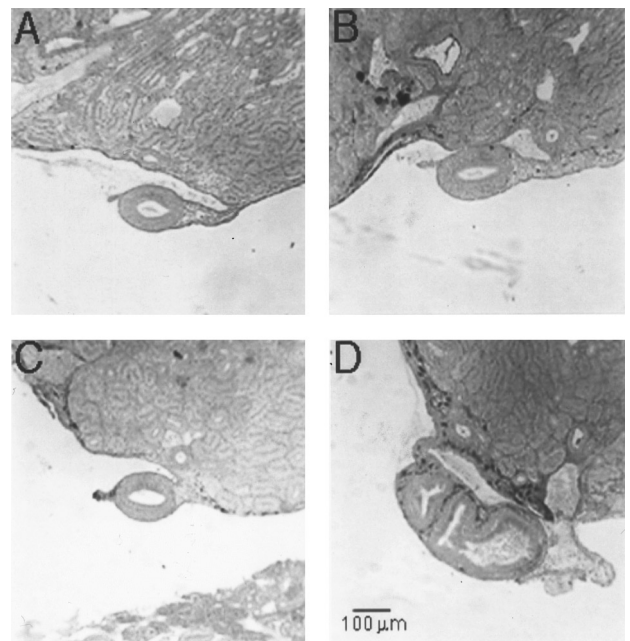


FIG. 4. Effect of 0.5  $\mu\text{g}$  of estrone (B), estradiol-17 $\beta$  (C), and estradiol (D) on oviduct development in the red-eared slider turtle (*Trachemys scripta*). All hormone-treated individuals were from eggs incubated at 26.0°, an all-male producing temperature and hormone treated during the middle of the temperature-sensitive period. The oviduct from a temperature-induced female hatchling (A) from an egg incubated at 31°, an all-female incubation temperature, and treated with ethanol shown for comparison.

17 $\beta$ , and estriol at an incubation temperature that normally produces only males, estriol was more potent than estrone and estradiol-17 $\beta$ , resulting in a greater number of females at a lower dosage; there was approximately a 15-fold increase in the logistic regression coefficient with estriol compared to that of estrone and estradiol-17 $\beta$ . Although estriol was very potent at an all male-producing temperature, there was no evidence of synergism with incubation temperature. Estrone had a high level of synergism with temperature from an all male-producing to a male-biased incubation temperature, the regression coefficient increased approximately 24-fold from 26 to 28.8° to a potency comparable to that of estriol. Estradiol-17 $\beta$  had a low degree of synergism with the lower incubation temperature but the regression coefficients continued to increase steadily with increasing temperature, having the strongest feminizing effect at 29°.

A possible explanation of this finding is that both estrone and estriol bind to the estrogen receptor but, unlike estradiol-17 $\beta$ , the cooperative interaction of the estrogen receptor with estrone and estriol is reversible and dependent upon temperature over a range of 0–30° (Sasson and Notides, 1983a,b). Changes in synergism with increasing temperature and different hormones may be due to changes in the concentration of the estrogen receptor with incubation temperature, changes in the cooperativity of hormone binding with temperature, and/or changes in affinity of the estrogen receptor with temperature. These changes may also be due to changes in the relative levels of different steroidogenic enzymes with incubation temperature which would affect endogenous concentrations of estrogens.

The hypertrophic effects of estrogens on vertebrate oviducts are well documented (reviewed by O'Malley *et al.*, 1975; Schimke *et al.*, 1975). Administration of high dosages (100  $\mu$ g) of estradiol-17 $\beta$ , as well as the synthetic estrogen-related compounds diethylstilbestrol and norethindrone, to eggs incubating at a male-producing temperature result in agenesis of the caudal portion and hypertrophy of the cranial portion of the Müllerian duct (Wibbels and Crews, 1992; see also Pieau, 1969, 1970, 1974). Ten micrograms of estradiol-17 $\beta$  results in individuals having full-length Müllerian ducts that are hypertrophied, whereas 1.0  $\mu$ g of both estradiol-17 $\beta$  and norethindrone produce individuals with normal Müllerian ducts (Wibbels and

Crews, 1992). The ability of exogenous estrogen to block the normal atrophy of the Müllerian ducts has also been observed in birds (Stoll *et al.*, 1987; 1990; MacLaughlin *et al.*, 1983; Hutson *et al.*, 1985; Osamu and Hutson, 1988). At the dosages used in the present study, females resulting from both estrone and estradiol-17 $\beta$  treatment had normal appearing oviducts, but estriol, even at a dosage of 0.1  $\mu$ g, stimulated abnormal oviduct development. Thus, estriol had greater potency on accessory sexual structures as well as the gonads.

It is clear from these data that the response machinery for female development is present and functional at an all-male producing incubation temperature. Since females are not normally produced at 26°, it underscores the importance of the hormonal milieu as the major switch and suggests that there may be little or no endogenous estrogen present at an all-male producing incubation temperature.

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