

The Relative Effectiveness of Androstenedione, Testosterone, and Estrone, Precursors to Estradiol, 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*; androgen is involved in male sex determination and estradiol in female sex determination. Administration of exogenous estradiol and its agonists to eggs incubating at a male-producing temperature can overcome the effect of temperature and result in all-female offspring. Exogenous testosterone will also result in some female hatchlings if administered to eggs incubated at a male-producing temperature, an effect due to the aromatization of testosterone to estradiol. This study demonstrates that in the red-eared slider, androstenedione, the precursor to both testosterone and estradiol, has a similar effect. In addition, both testosterone and androstenedione synergize with incubation temperature to exert a greater effect at intermediate incubation temperatures that normally produce mixed sex ratios, indicating that as with estradiol, androstenedione and testosterone are involved in the final common pathway of sex determination in this species. At the single dosage administered, estrone and estradiol produced all females at a male-producing incubation temperature. © 1995 Academic Press, Inc.

Temperature-dependent sex determination (TSD) is well established in many reptiles. For example, depending upon the species, turtles with TSD may exhibit one of several different patterns: low incubation temperatures produce males, high incubation temperatures produce males, and medium incubation temperatures produce males with females being produced at both extremes (Bull, 1980; Pieau *et al.*, 1994). In the red-eared slider turtle, *Trachemys scripta*, incubation at 20–28.8° produces all males and 29.5–35° produces all females; only a narrow (<1°, intermediate, range of incubation temperatures produces both sexes (Crews *et al.*, 1994). Incubation temperature exerts its effect only during the midtrimester of development (Wibbels *et al.*, 1991a). Sex steroid hormones appear to be the physiological equivalent of incubation temperature and the proximate trigger for female and male sex determination in the red-eared slider. Estrogen is involved in female sex

determination, whereas nonaromatizable androgen appears to be involved in male sex determination (Bull *et al.*, 1988; Crews *et al.*, 1991, 1994; Wibbels and Crews, 1994, 1995; Wibbels *et al.*, 1991b). The morphological changes occurring in response to exogenous estrogen are indistinguishable from the changes induced by incubation temperature (Wibbels *et al.*, 1993), and studies with other TSD reptiles indicate that estrogen-determined females are similar endocrinologically (Rhen *et al.*, 1995) and as fecund (Crews *et al.*, 1994) as temperature-determined females.

Administration of exogenous testosterone (T) to eggs incubated at a female-producing temperature is without effect (Gutzke and Bull, 1986; Crews *et al.*, 1989; Wibbels and Crews, 1995), but if administered to eggs incubating at a male-producing temperature, some females will be produced (Crews and Bergeron, 1994; Crews *et al.*, 1989; Gutzke and Bull, 1986;

Pieau, 1974). This "paradoxical" effect is due to the conversion of the T to estrogen, as administration of an aromatase inhibitor in addition to T to eggs incubating at male-producing temperature results in only male offspring (Crews and Bergeron, 1994); administration of aromatase inhibitor alone at a female-producing incubation temperature results in male offspring (Dorizzi *et al.*, 1994; Crews and Bergeron, 1994; Rhen and Lang, 1994; Wibbels and Crews, 1994). Administration of nonaromatizable androgens such as dihydrotestosterone (DHT) or synthetic ligands such as R1881 will induce significantly more males at intermediate incubation temperatures that normally produce mixed sex ratios, but not at an incubation temperature that produces only females (Crews *et al.*, 1994; Wibbels and Crews, 1994; Wibbels *et al.*, 1992).

After hydroxylation in the 19 position, androstenedione (AE) is the immediate precursor to estrone (E1) and T, both of which are converted to estradiol (E2). The most thorough investigation of steroid metabolism during embryogenesis has been conducted by Pieau and co-workers on the pond turtle (*Emys orbicularis*). Pieau *et al.* (1982) report higher levels of AE and DHT in embryonic testes compared to those in ovaries. Studies utilizing radioactive precursors indicate that the differentiating gonads of the pond turtle can produce E1 and E2 (Desvages and Pieau, 1991), and radioimmunoassay reveals that during the temperature-sensitive period, gonads at a female-producing incubation temperature have higher estrogen content than gonads at a male-producing temperature (Dorizzi *et al.*, 1991). Incubation of gonadal explants from embryonic pond turtles with radioactively labeled AE yields little estrogen (Desvages and Pieau, 1991), suggesting that in this species the preferred metabolic pathway is from T to E2.

The purpose of the present experiment was to compare the effects of precursors of estradiol (AE, T, and E1) on sex determination of the red-eared slider. In addition, different incubation temperatures were utilized to determine if there is a synergism between incubation tem-

perature and AE and T as occurs between incubation temperature and E2. Such a result would suggest that these hormonal products lie in a common pathway with incubation temperature.

MATERIALS AND METHODS

Freshly laid eggs were obtained commercially (Robert Kliebert, Hammond, LA). After transport to our laboratory, eggs were held at room temperature until viability was established by candling. They were then placed in containers with moistened vermiculite (vermiculite:water, 1:1) and the containers placed in reach-in incubators (Precision) programmed to provide a constant temperature ($\pm 0.1^\circ$). Previous studies with this species indicate that a continuous incubation temperature of 26.0° produces all male hatchlings whereas an incubation temperature of 31.0° produces all female hatchlings; intermediate incubation temperatures produce varying sex ratios, with 29.0° resulting in approximately 85% male and 29.2° resulting in approximately 50% male (Crews *et al.*, 1994). Embryonic development was monitored by candling eggs and by dissecting two to four eggs approximately twice a week to verify specific developmental stages, based on criteria described by Wibbels *et al.* (1991a). All eggs were randomized into ethanol control and ligand treatment groups and incubated at 26.0 , 29.0 , or 29.2° .

Eggs in control groups received a single treatment consisting of $5\mu\text{l}$ of 95% ethanol. Eggs from experimental groups received a single treatment at the beginning of the temperature-sensitive period (stage 17) of a specific ligand dissolved in $5\mu\text{l}$ of 95% ethanol. Steroid hormones (AE, T, E1, and E2) were obtained from Sigma. The dosages chosen for each ligand were based on previous studies with turtles (Gutzke and Bull, 1986; Bull *et al.*, 1988; Crews *et al.*, 1989, 1991) and were as follows: T and AE, 10, 50, and 100 μg ; E1 and E2, 5 μg . All of the treatments were applied topically to the vascularized portion of the upper shell (Crews *et al.*, 1991). The total number of eggs treated varied according to treatment (ethanol, 148 (78 for AE and 70 for T); AE, 258; T, 244; E1, 30; and E2, 30) with group size ranging from 22 to 32 and averaging 27 per group.

After receiving treatment, all of the eggs were placed back into their incubators until they had hatched. Turtles were sacrificed by decapitation approximately 2-4 weeks after hatching. Gonadal sex and developmental status of the Müllerian ducts were assessed macroscopically by examination of the reproductive tracts under a dissection microscope. The gonads of hatchling red-eared sliders are relatively well differentiated and, with rare exception, appear distinctly testicular or ovarian when viewed under a dissection microscope. Ovaries are long and flat, whereas testes are shorter, more round, and have visible seminiferous tubules (Crews *et al.*, 1991; Wibbels *et al.*, 1991a). The gonads of three to five individuals from each experimental group were processed for histological examination of the gonad to confirm sex assignment. In all of the instances the histolog-

ical assessment of sex coincided with the macroscopic assignment of sex. The oviducts were examined and scored as either absent, regressed but visible, or normal (as in a typical female hatchling). The presence and development of the phallus was also noted. Normally, in the female hatchling the cloaca is without pigment and the phallus development is rudimentary if present at all, 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. As found in previous studies (e.g., Wibbels *et al.*, 1992), the administration of exogenous DHT has either an accelerated or an extreme effect on phallus development. Accelerated development is indicated by a hypertrophied and more solid shaft with well-developed caudal portion; the genital skin also has a greater amount of pigmentation than typically seen in a normal male and is visible through the cloacal wall. High dosages commonly lead to a greatly hypertrophied structure that has a swollen and misshapen appearance and is everted through the cloacal opening; the area is heavily pigmented.

Synergism occurs when the effect of two factors together have a greater effect than the sum of their separate effects. Synergism between incubation temperature and hormone treatment is consistent with the hypothesis that these factors work in the same biochemical pathway for sex determination. Logistic regression (Sokal and Rohlf, 1981; Chatterjee and Price, 1978) of sex ratio (males:females) was used to test for the effects of incubation temperature, hormone treatment, temperature by hormone interaction, and synergism between temperature and hormone. Specifically, the effect of each hormone (either AE or T) within each temperature

treatment was determined using polynomial logistic regression; a backward stepwise procedure was used to reduce the model to include only those regression coefficients that explained a significant amount of variation in the sex ratio data. Next, Gabriel's (1978) method was used to compare regression coefficients (i.e., b_0 , b_1 , b_2 , or b_3) by computing lower and upper comparison limits; coefficients are significantly different if their intervals do not overlap (Sokal and Rohlf, 1981). Synergism between incubation temperature and hormone treatment is present if b_1 at intermediate temperatures (29 and 29.2°) is 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 the baseline temperature.

RESULTS

The percentages of female hatchlings in each experimental group as a result of exogenous AE or T treatment are shown in Fig. 1. In the AE experiment, the sex ratios of the groups exhibited significant variation ($\chi^2 = 466.9$, $df = 12$, $P < 0.0001$). Ethanol treatment alone had no significant effect on the hatchling sex ratio as previously established for the red-eared slider turtle (Crews *et al.*, 1994; Wibbels *et al.*, 1991b). In the AE experiment, an incubation temperature of 26.0° produced all males (male:female sex

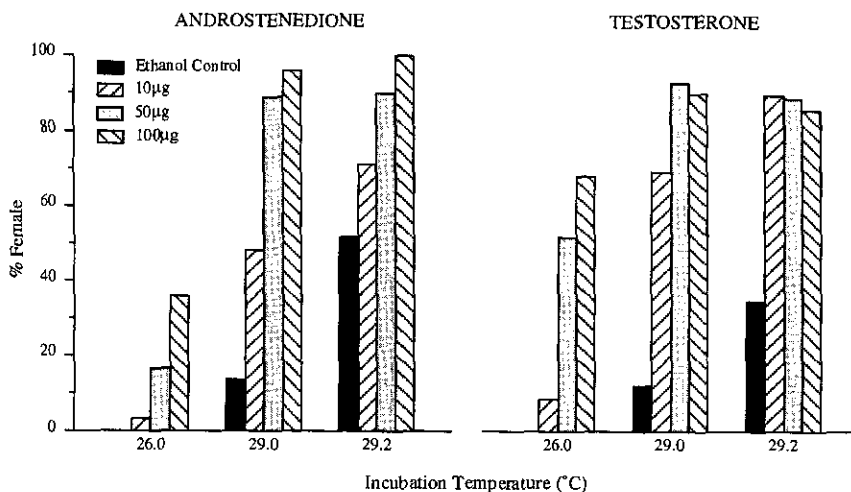


FIG. 1. Effect of varying dosages of exogenous androstenedione or testosterone on sex determination in the red-eared slider turtle (*Trachemys scripta*). Shown is the percentage of female hatchlings produced at each incubation temperature. All of the eggs were spotted with hormone in alcohol (shaded bars) or with alcohol alone (black bars) during the temperature-sensitive period. Sample size average 27 individuals, ranging from 22 to 32; total number of individuals depicted, 336 for androstenedione and 314 for testosterone.

ratio, 27:0), whereas 29.0° produced a 19:3 sex ratio and 29.2° produced a 14:15 sex ratio; the sex ratios produced at these temperatures were significantly different from each other (Table 1, b_0 's all significantly different from each other). There was a dose-response relationship with increasing dosages of AE resulting in increasing proportions of females at each incubation temperature (Table 1, all b_1 's significantly greater than 0). Finally, the effect of AE was temperature-sensitive; that is, the effect of AE compared to the ethanol control produced greater numbers of females at 29.0° than at 26.0° (Table 1, b_1 at 29.0° significantly greater than b_1 at 26.0°). The effect of AE at 29.2° was intermediate to its effects at 26.0 and 29.0° (Table 1, b_1 at 29.2° not detectably different from b_1 at 26.0 or at 29.0°). The ceiling effect, or approach of sex ratio to 100% female, at 29.0° is shown by the significant negative b_2 regression coefficient at that temperature (Table 1).

In the T experiment, the sex ratios of the groups exhibited significant variation ($\chi^2 = 422.2$, $df = 12$, $P < 0.0001$). Again, ethanol treatment alone had no detectable effect on the hatchling sex ratio in this experiment. An incubation temperature of 26.0° produced all males (male:female sex ratio, 22:0), whereas 29.0° produced a 22:3 sex ratio and 29.2° produced a 15:8 sex ratio; these temperatures produced increasing proportions of females (Table 2, b_0 's

increase with increasing incubation temperature). There was also a dose-response relationship for T with increasing dosages of T resulting in increasing proportions of females at each incubation temperature (Table 2, all b_1 's significantly greater than 0). The effect of T was also temperature-sensitive; T produced greater numbers of females at 29.0° than at 26.0° (Table 2, b_1 at 29.0° significantly greater than b_1 at 26.0°). The effect of T at 29.2° was intermediate to its effects at 26.0 and 29.0°. A ceiling effect, or asymptotic effect of T at higher doses, is shown by the significant negative b_2 regression coefficients at all of the incubation temperatures (Table 2, b_2 's less than 0). There are significant third-order regression coefficients at 29 and 29.2° (Table 2, b_3 's greater than 0). There was a significant incubation temperature by T interaction in the full model ($\chi^2 = 8.11$, $df = 1$, $P = 0.0044$). This was due to synergism between temperature and T at the lowest dosage of T and a lower number of females than expected at the highest dosage of T at 29.2° (based on additive and/or synergistic models for the effect of T and incubation temperature).

Administration of both E1 and E2 resulted in 100% female hatchlings at an otherwise all-male-producing incubation temperature ($\chi^2 = 74.0$, $df = 1$, $P = 0.0001$).

There were no histological differences detected in the testicular or ovarian nature of the

TABLE 1
SEPARATE REGRESSION RESULTS FOR THE EFFECT OF INCREASING ANDROSTENEDIONE CONCENTRATIONS ON THE HATCHLING SEX RATIO IN THE RED-EARED SLIDER TURTLE (*Trachemys scripta*) AT THREE INCUBATION TEMPERATURES

Regression coefficient	Incubation temperature		
	26.0	29.0	29.2
b_0	-3.64 ± 0.67^a ($\chi^2 = 29.17$, $P < 0.0001$) [$t = -4.78$, $u = -2.50$]	-1.36 ± 0.41^b ($\chi^2 = 11.1$, $P = 0.0009$) [$t = -2.06$, $u = -0.66$]	0.2039 ± 0.2920^c ($\chi^2 = 0.49$, $P = 0.49$) [$t = -0.2925$, $u = 0.7003$]
b_1	0.03171 ± 0.00837^a ($\chi^2 = 14.37$, $P = 0.0002$) [$t = 0.01748$, $u = 0.04594$]	0.10416 ± 0.03144^b ($\chi^2 = 10.97$, $P = 0.0009$) [$t = 0.05072$, $u = 0.15760$]	0.04637 ± 0.01308^{ab} ($\chi^2 = 12.57$, $P = 0.0004$) [$t = 0.02414$, $u = 0.06860$]
b_2	ns	-0.0006489 ± 0.0003183 ($\chi^2 = 4.16$, $P = 0.042$)	ns

Note. Regression coefficients ± 1 standard error with χ^2 and probability values in parentheses; there is 1 df for each regression coefficient. Regression coefficients with different superscript letters 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; ns, not significant.

TABLE 2
SEPARATE REGRESSION RESULTS FOR THE EFFECT OF INCREASING TESTOSTERONE CONCENTRATIONS THE HATCHLING SEX RATIO IN THE RED-EARED SLIDER TURTLE (*Trachemys scripta*) AT THREE INCUBATION TEMPERATURES

Regression coefficient	Incubation temperature		
	26.0	29.0	29.2
b_0	-3.86 ± 0.96^a ($\chi^2 = 16.37$, $P = 0.0001$) [$t = -5.49$, $u = -2.23$]	-1.99 ± 0.62^{ab} ($\chi^2 = 10.48$, $P = 0.001$) [$t = -3.04$, $u = 0.94$]	-0.61 ± 0.44^b ($\chi^2 = 1.94$, $P = 0.16$) [$t = -1.36$, $u = 0.14$]
b_1	0.1128 ± 0.0345^a ($\chi^2 = 10.71$, $P = 0.001$) [$t = 0.0542$, $u = 0.1715$]	0.3484 ± 0.0996^b ($\chi^2 = 12.22$, $P = 0.0005$) [$t = 0.1791$, $u = 0.5177$]	0.2855 ± 0.0892^{ab} ($\chi^2 = 10.24$, $P = 0.001$) [$t = 0.1339$, $u = 0.4371$]
b_2	-0.000667 ± 0.000275^a ($\chi^2 = 5.9$, $P = 0.015$) [$t = -0.001134$, $u = -0.00020$]	-0.007242 ± 0.00277^b ($\chi^2 = 6.85$, $P = 0.0089$) [$t = -0.01195$, $u = -0.00253$]	-0.00704 ± 0.00255^b ($\chi^2 = 7.6$, $P = 0.0058$) [$t = -0.01138$, $u = -0.00271$]
b_3	ns	$0.00004176 \pm 0.0000186^a$ ($\chi^2 = 5.05$, $P = 0.025$) [$t = 0.000010$, $u = -0.000073$]	$0.00004385 \pm 0.0000171^a$ ($\chi^2 = 6.59$, $P = 0.01$) [$t = 0.000015$, $u = 0.000073$]

Note. Regression coefficients ± 1 standard error with χ^2 and probability values in parentheses; there is 1 *df* for each regression coefficient. Regression coefficients with different superscript letters 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; ns, not significant.

gonads from hormone-treated hatchlings versus male and female control hatchlings; hermaphroditic (ovotestes) gonads were not observed.

As shown in Table 3, the presence or absence of oviducts was generally consistent, with only a few of the gonadal males retaining oviducts at the higher dosage of T. In addition to noting the Müllerian ducts, abnormal phallus development was observed in all but two of the treatment groups. In these instances phallus development was accelerated and similar to that expected for an adult male or everted, whereby the phallus was hypertrophied, extruding through the cloacal opening. In all of the other instances phallus development was as expected for male or female hatchlings.

DISCUSSION

The biosynthesis of T and E2 from cholesterol involves the action of several enzymes. The initial rate-limiting step is the conversion of the C_{27} steroid, cholesterol, to the C_{21} steroid, pregnenolone, which is catalyzed by the cytochrome P450 enzyme, cholesterol side-chain cleavage. Pregnenolone is then converted to progesterone by the action of 3β -hydroxysteroid dehydrogenase (3β -HSD). The cytochrome P450 17α -hydroxylase/ C_{17-20} lyase is

involved in formation of AE, the immediate precursor of T. Androstenedione in turn is converted by aromatase to E1 and by 17β -HSD to T. Testosterone is converted by the cytochrome P450 enzyme aromatase to E2 whereas E1 is converted by 17β -HSD to E2. Testosterone is also converted by 5α -reductase to 5α -dihydrotestosterone (5α -DHT), which in turn is converted by $3\alpha/3\beta$ steroid ketoreductases to 3α - and 3β -androstenediols.

Hydroxysteroid dehydrogenases, reductase, and aromatase enzymes are present in the urogenital system of embryos of TSD species (Merchant-Larios and Villalpando, 1990; Pieau *et al.*, 1994; Smith and Joss, 1994; Thomas *et al.*, 1992), suggesting steroid biosynthesis. Standard histochemical methods have been used to assess the activity of 3β - and 17β -HSD enzymes before, during, and after sex determination at both male- and female-producing incubation temperatures in the red-eared slider turtle (Thomas *et al.*, 1992). Male- and female-producing incubation temperatures result in different patterns of HSD activity in the adrenal and mesonephros during development. Significantly, reaction product was not observed in the genital ridge in embryos before the temperature-sensitive period at either incubation temperature, nor was it apparent in the differentiating gonads in embryos during the temperature-

TABLE 3
EFFECT OF ADMINISTRATION OF VARIOUS DOSAGES OF EXOGENOUS ANDROSTENEDIONE (AE) AND TESTOSTERONE (T)
DURING INCUBATION AT DIFFERENT TEMPERATURES ON THE DEVELOPMENT OF PRIMARY, ACCESSORY, AND SECONDARY
SEXUAL STRUCTURES IN THE RED-EARED SLIDER TURTLE (*Trachemys scripta*)

Hormone	Incubation temperature	Dosage (μg)	n	Ovaries	Oviducts	Testes	Phallus	
							Expected development	Accelerated development
AE	26.0	0	27	0	0	27	27	0
		10	32	1	1	31	1	31
		50	30	5	5	25	4	26
		100	28	10	10	18	1	27
	29.0	0	22	3	3	19	22	0
		10	27	14	14	13	27	0
		50	27	24	24	3	24	3
		100	23	23	23	0	1	6
	29.2	0	29	15	15	14	29	0
		10	31	22	22	9	3	28
		50	30	27	27	3	27	3
		100	30	30	30	0	0	14
T	26.0	0	22	0	0	22	22	0
		10	24	2	2	22	24	0
		50	27	14	14	13	27	0
		100	25	17	20*	8	14	8
	29.0	0	25	22	22	3	25	0
		10	26	18	18	8	26	0
		50	28	26	27*	2	21	6
		100	30	27	30*	3	15	9
	29.2	0	23	8	8	15	23	0
		10	29	26	26	3	n.r.	n.r.
		50	27	24	26*	3	17	8
		100	28	24	24	4	n.r.	n.r.

Note. Numbers of individual treated hatchlings which showed sex-specific structures as indicated. Zero dosage indicates ethanol control. Normally, female hatchlings as indicated by the presence of ovaries and oviducts have no evident phallus, whereas gonadal male hatchlings (testes) have normal rudimentary phallus development; see text for detailed description of phallus development. *Indicates groups in which some gonadal males retained Müllerian ducts; n.r. indicates that phallus development was not recorded for these groups.

sensitive period; the only activity detected in the gonad was observed after the temperature-sensitive period. This pattern has also been seen in the Olive Ridley sea turtle, *Lepidochelys olivacea* (Merchant-Larios and Villalpando, 1990), and the saltwater crocodile, *Crocodylus porosus* (Smith and Joss, 1994), but not in the pond turtle (Pieau *et al.*, 1994). This suggests that in the red-eared slider, the tissues proximate to the gonad, and not the gonad itself, produce steroid hormones before and during the sex-determining period. Present studies are directed toward measuring aromatase and reductase during gonadal differentiation in this species.

In the pond turtle (*E. orbicularis*), steroid metabolism studies utilizing radioactive precursors indicate that the differentiating gonads of the pond turtle can produce E1 and E2 (Desvages and Pieau, 1991), and radioimmunoassay reveals that during the temperature-sensitive period, gonads at a female-producing incubation temperature have higher estrogen content than gonads of embryos from a male-producing incubation temperature (Desvages and Pieau, 1992; Dorizzi *et al.*, 1991). Further, aromatase activity is very low in undifferentiated gonads; it remains low in embryos at a male-producing temperature but increases exponentially in embryos at a female-producing temperature (re-

viewed in Pieau *et al.*, 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 levels. These changes do not appear to be due to temperature modulation of aromatase activity, but rather to temperature-induced increases in expression of the aromatase gene.

In examining the gonadal sex of the hatchling turtles, AE and T, both precursors in the steroidogenic pathway for E2 production, showed a synergistic effect with incubation temperature. Because a synergism between E2 and incubation temperature also occurs in this species (Wibbels *et al.*, 1991b), it is not surprising that these androgens display a similar effect on sex determination. The synergism between E2 and incubation temperature, however, appears to be much stronger than that between AE and T and temperature. The dosage of E2 (5 μg) used produced all females at a male-producing incubation temperature (26°), whereas 10 μg of AE or T resulted in only a slight increase in the number of females, with increasing females at higher dosages. In this experiment, neither AE nor T produced 100% females at 26°. If estrogen is the proximate signal for female differentiation and aromatase expression is temperature-dependent, an increase in incubation temperature (as in this experiment), along with an increase in the estrogen precursors (AE and T), should result in a higher percentage of female offspring than expected based on an additive model. Thus, the most parsimonious model is one in which sex steroids and incubation temperature share a common pathway (Wibbels *et al.*, 1991b). Many estrogens, estrogen agonists, and aromatizable androgens act to feminize embryos incubated at male-producing incubation temperatures (reviewed in Crews *et al.*, 1994; Wibbels *et al.*, 1994; Pieau *et al.*, 1994; but also see Rhen and Lang, 1994). In addition, there is notable evidence that aromatase expression is temperature-dependent (Dorizzi *et al.*, 1994; D. Crews and J. M. Bergeron, unpublished data). Also consistent with this model, aromatase inhibitors produce males at female-biased incubation temperatures and also block the feminizing

effects of aromatizable androgens (Crews and Bergeron, 1994; Rhen and Lang, 1994).

When comparing the effects of T to those of AE at 26°, an incubation temperature that normally produces only males, T appears to be more potent than AE, resulting in a greater number of females at the lower dosage. Regarding the increase in number of females due to dosage of each hormone (regression coefficients b_1 ; Tables 1 and 2), there is approximately a threefold increase with T compared to that of AE. This trend is also evident in the comparison of the lower dosages at the male-biased temperatures (b_1 for T is approximately 3 \times that of AE). The potency of T when compared to AE is also shown by the ceiling effect in the higher dosages of T. In this system, the efficacy of T in producing females may be greater than that of AE simply because of a difference in their rates of conversion to estrogens. For example, aromatase may have a higher affinity for T than for AE, which could result in different rates of conversion to E1 and E2, respectively.

At the dosage used in this experiment (5 μg), E1 and E2 produced all females at a male-producing incubation temperature. It is possible that both estrogens act through the estrogen receptor to induce transcription of the genes responsible for female sex determination and inhibit the genes responsible for male sex determination (Crews *et al.*, 1994). Further dose-response studies to compare E1 and E3 (estriol) sensitivity to E2 sensitivity are being conducted to examine this question.

In addition to the gonads, we examined the development of the phallus in the hatchling turtles. Especially in the higher dosages of the androgens AE and T, increasingly higher numbers of accelerated male secondary sex structures were observed. As shown in Wibbels *et al.* (1992), DHT is active in the formation of phallus in the red-eared slider and high dosages of DHT result in accelerated and even hypertrophied phallic development. This is consistent with the regulation of development of the external genitalia by 5 α -DHT in mammals (Russell and Wilson, 1994). The present results of accelerated and abnormal phallus development

with both T and AE administration indicate that the precursors are being converted to 5 α -DHT. Alternatively, 5 α -DHT may not be the proximate trigger for phallus development. Rather, it could be the androgen-androgen receptor (AR) interaction that regulates phallus development through transcriptional control of specific growth factors. Here, the precursors AE and T may themselves be acting with the AR, in turn regulating growth of secondary sexual structures.

Recent research indicates that more extreme temperatures have a greater potency in exerting their effect (Bull *et al.*, 1990; Wibbels *et al.*, 1991b). In addition, incubation temperature can synergize with exogenous hormone treatment; eggs incubated at male-producing temperatures close to the temperature threshold for female development are more sensitive to estrogen (Wibbels *et al.*, 1991b). The present study indicates this extends to the aromatizable precursors of estradiol, T and AE, as well.

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