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Testicular Steroid Biosynthesis by the Green Lizard *Lacerta viridis*

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Testes from the green lizard *Lacerta viridis* were incubated with [^{3}H]pregnenolone or [^{3}H]testosterone and the products were identified by chromatography, microchemical reaction, and crystallisation to constant specific activity or isotope ratio. The major metabolites of pregnenolone were testosterone (40.8%), androstenedione (5.5%), 5α-androstane-3β,17β-diol (4.4%), and 5α-pregnane-3β,17α,20ξ-triol (15.2%). Androstenedione was the only identifiable metabolite (4.8%) of testosterone.

Although testosterone[1] is the major testicular androgen in all of the species of mammals so far investigated, there is considerable variation in the nature of the testicular hormone amongst the nonmammalian vertebrates. In teleost fish testosterone, 11-oxo- and 11β-hydroxytestosterone are the major androgens (Arai and Tamaoki, 1967; Idler and MacNab, 1967), whereas in elasmobranch fish testosterone and its sulphate are the only identifiable androgens (Darrow and Fletcher, 1972; Fletcher *et al.*, 1969; Idler and Truscott, 1966; Kime, 1978; Simpson *et al.*, 1964). In anuran amphibians dihydrotestosterone is a major testicular hormone (Kime and Hews, 1978; Muller, 1976, 1977; Ozon *et al.*, 1964; Ozon and Stocker, 1974), but in the urodele amphibians and reptiles testosterone is the only testicular androgen so far identified. (Ozon, 1967; Lupo di Prisco *et al.*, 1972; Muller, 1976; Rivarola *et al.*, 1968). Very few species, however, have been examined within each of these classes and generalisations based on only two or three species could be misleading. Within the Reptilia, for example, testicular steroids have been isolated and rigorously identified only in the snakes *Natrix sipedon pictiventris* (Callard, 1967) and *Naja naja* (Lofts and Choy, 1971; Tam and Phillips, 1969). In both species, testosterone was the major *in vitro* metabolite of pregnenolone or progesterone, but in the lizard *Lacerta sicula* endogenous testosterone was found to be present only as a conjugate (Lupo di Prisco *et al.*, 1967). In order to extend this knowledge of reptilian testicular steroid biosynthesis we have examined the metabolism of pregnenolone and testosterone by testes of the green lizard *Lacerta viridis*.

[1] Steroid nomenclature: testosterone, 17β - hydroxy - 4 - androsten - 3 one; 11 - oxotestosterone, 17β - hydroxy - 4 - androstene - 3,11 - dione; 11β - hydroxytestosterone, 11β,17β - dihydroxy - 4 - androsten - 3 - one; dihydrotestosterone, 17β - hydroxy - 5α - androstan - 3 - one; epiandrosterone, 3β - hydroxy - 5α - androstan - 17 - one; androstenedione, 4 - androstene - 3,17 - dione; pregnenolone, 3β-hydroxy - 5 - pregnen - 20 - one; progesterone, 4 - pregnene - 3,20 - dione.

MATERIALS AND METHODS

Materials. [4-^{14}C]Testosterone (58.2 mCi/mmol), [1,2,6,7-^{3}H]testosterone (100 Ci/mmol), and [7-^{3}H] pregnenolone (18.6 Ci/mmol) were purchased from the Radiochemical Centre, Amersham, and their purity was checked in two chromatographic systems before use. [4-^{14}C]Androstenedione was prepared by oxidation of [4-^{14}C]testosterone. Reference steroids were obtained from either Steraloids or Sigma.

Sexually mature specimens of green lizards, captured in Europe, were obtained from a commercial supplier in May and kept alive in a vivarium at 20 to

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25° in this Department until July, when they were sacrificed by decapitation.

Incubation. For each incubation, 250 mg of finely chopped testes from four lizards was used. In 20-ml incubation vials, 10 μCi of the radioactive steroid was dried, 5 ml of Kreb's Ringer bicarbonate physiological medium was added, and the flask was agitated for 10 min before addition of the tissue. Incubations were carried out for 3 hr at 22° under 95% O_2/5% CO_2. No cofactors were added.

Isolation of metabolites. The extraction of metabolites from the incubation medium and β-glucuronidase and acid hydrolysis of the conjugates were carried out as previously described (Kime, 1978; Kime and Hews, 1978). One hundred micrograms each of testosterone, androstenedione, dihydrotestosterone, and 5α-androstane-3β,17β-diol carriers were added to the aqueous phase prior to extraction of both free steroids and hydrolysed conjugates.

Identification of metabolites. Metabolites were separated by paper and thin-layer chromatography carried out as previously described (Kime, 1978; Kime and Hews, 1978). The tlc systems used were: System I, chloroform–methanol (98:2); System II, chloroform; System VI, chloroform–methanol (95:5). Metabolites were identified by their isopolarity with carrier steroids, isopolarity of at least one chemical derivative with the authentic derivative, and finally by crystallisation of a derivative to either constant isotope ratio or constant specific activity with the authentic derivative. The criteria for identification are fully described in an earlier communication (Kime and Hews, 1978).

RESULTS

When lizard testes were incubated with [^{3}H]pregnenolone and the products were chromatographed on paper in the Bush B_3 system, four main peaks of radioactivity were resolved, corresponding to carrier androstenedione, testosterone, 5α-androstane-3β,17β-diol, and 5α-pregnanetriol. The androstenedione fraction was further resolved by tlc in System I into two peaks with the polarities of androstenedione and pregnenolone or dihydrotestosterone. Androstenedione was identified by reduction to testosterone and crystallisation with authentic steroid to constant isotope ratio. The second peak, on acetylation, gave a peak of activity isopolar in tlc System II with dihydrotestosterone acetate, but crystallisation with this compound failed to give a constant specific activity. This compound (2.1% yield) was not further investigated. The androstanediol fraction gave a single peak of activity on tlc in System I, but on acetylation only 30% was convertible into a product isopolar with 5α-androstane-3β,17β-diol diacetate. This compound was identified by admixture with the authentic acetate and crystallisation to constant specific activity. The nonacetylable metabolite could not be further identified. The triol fraction, which was isopolar with authentic triol in tlc System VI, gave on acetylation a product isopolar in tlc System II with pregnanetriol diacetate. Periodate oxidation of the suspected triol gave a compound which by tlc in System I and crystallisation to constant specific activity was identified as epiandrosterone. The original triol was thus identified as 5α-pregnane-3β,17α,20ξ-triol, but the high cost of authentic material prevented further elucidation of the configuration at C-20.

Incubation of lizard testes with [^{3}H] testosterone gave a product which on paper chromatography gave two peaks of radioactivity, the major one of which corresponded to carrier testosterone and was identified as previously described. The minor peak was resolved on tlc into two peaks with the polarities of androstenedione and dihydrotestosterone. Androstenedione was positively identified by reduction and crystallisation, but the more polar peak (2% yield) which on acetylation gave a product of similar polarity to dihydrotestosterone acetate in tlc System II failed to give a constant specific activity when this acetate was crystallised with authentic dihydrotestosterone acetate.

No activity was found in the conjugate fractions from either incubation.

DISCUSSION

Our results show that in the green lizard *Lacerta viridis* the main testicular steroids *in vitro* are testosterone and andros-

TABLE 1
IDENTIFICATION OF INCUBATION PRODUCTS BY CRYSTALLISATION TO CONSTANT SPECIFIC ACTIVITY OR ISOTOPE RATIO[a]

	Initial	First crystallisation	Second crystallisation	Third crystallisation	Fourth crystallisation	Percentage yield
Incubation of [^{3}H]pregnenolone						
Androstenedione[b]	4.48	4.56	4.98	4.59	4.78	5.5
Testosterone[b]	13.7	14.2	13.4	13.9	14.7	40.8
5α-Androstane-3β,17β-diol[c]	103	90.2	81.4	77.6	77.7	4.4
5α-Pregnane-3β, 17α,20ξ-triol[c]	521	502	449	454	416	15.2
Incubation of [^{3}H]testosterone						
Androstenedione	3.39	3.14	3.23	3.17	3.54	4.8
Testosterone	12.8	12.7	12.4	12.3	13.2	71.6

[a] Crystallisation solvents: 5α-androstanediol deacetate was crystallised from acetone–hexane, aqueous methanol, aqueous acetone, and aqueous ethanol. All other compounds were crystallised from acetone–hexane, chloroform–hexane, aqueous methanol, and aqueous ethanol.

[b] Isotope ratio (^{3}H/^{14}C ratio of counts).

[c] Specific activity (counts per minute per milligram).

tenedione. This is in accord with previous work on *in vitro* testis biosynthesis in reptiles. Both testis and seminiferous tubules of the cobra (*Naja naja*) have been shown to convert progesterone into testosterone and androstenedione *in vitro* (Tam and Phillips, 1969; Lofts and Choy, 1971), and testosterone and progesterone were isolated from incubations of pregnenolone with testes of the water snake *Natrix sipedon pictiventris* (Callard, 1967). In *Lacerta sicula*, however, Lupo di Prisco *et al.* (1967) were able to isolate endogenous testosterone only as its conjugated form. We examined both glucuronide and solvolysable conjugates in our *Lacerta viridis* testis incubations but were unable to detect any radioactivity in either of these fractions. Testes of anuran amphibians rapidly convert testosterone into dihydrotestosterone (Kime and Hews, 1978) and this compound has been shown to be a major circulating androgen in these species (Muller, 1976, 1977). We were unable to detect dihydrotestosterone in the lizard but did detect two reduced metabolites of pregnenolone, 5α-androstane-3β,17β-diol and 5α-pregnane-3β,17α,20ξ-triol. The absence of the diol as a metabolite of testosterone indicates that in reptiles as in anuran amphibians there may be a pathway to 5α-reduced C-19 steroids which does not involve testosterone and in which pregnanetriol may be an intermediate.

Although more species should be investigated, results so far indicate that reptiles, like urodele amphibians, possess a pattern of testicular androgens very similar to that of mammals.

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