

### Steroid Hormones in the Testicular Tissue of *Gallus domesticus*

The presence of gonadal steroid hormones in birds has been studied by LAYNE et al.<sup>1</sup>, who found oestradiol-17 $\beta$ , oestrone and progesterone<sup>2</sup> in the ovary of the laying hen.

Researches in vitro by FEVOLD and EIK-NES have shown the presence of the enzymes which are needed for the main steps of steroid biosynthesis in the testicular tissue of the English sparrow *Passer domesticus*<sup>3</sup>.

The nature of the steroid hormones in testicular tissue of birds is still unknown; therefore we have analyzed material extracted from the testes of the cock *Gallus domesticus*.

From 15 mature animals, 278 g of fresh tissue were obtained, which gave 30 g of lyophilized tissue. The latter was extracted with organic solvent to give the free-steroid residue. The conjugated steroids were extracted after acid hydrolysis with 0.5N HCl in 50% dioxane for 24 h. Both free and conjugated steroids were separated into neutral and phenolic fractions by extraction with 1N NaOH. The residues were purified by partition between petroleum ether and 70% methanol followed by column chromatography and thin-layer chromatography. The identification of the steroids was done by UV-spectra, gas-liquid chromatography and conversion to derivatives.

Five steroids appeared to be present in the extract of the neutral free fraction: pregnenolone, progesterone, 20 $\beta$ -hydroxypregn-4-en-3-one, androstenedione and testosterone. Pregnenolone, androstenedione and testosterone were also identified respectively through acetylation, reduction with NaBH<sub>4</sub> and oxidation with CrO<sub>3</sub>.

The concentration of the steroids in the tissue determined from the gas-liquid chromatographic areas was the following: pregnenolone 127  $\mu$ g/kg; progesterone 66.5  $\mu$ g/kg; 20 $\beta$ -hydroxypregn-4-en-3-one 30.5  $\mu$ g/kg; androstenedione 2.4  $\mu$ g/kg; testosterone 35  $\mu$ g/kg.

The analysis of the neutral conjugated fraction did not show the presence of any steroids. No oestrogens were found in both free and conjugated phenolic fractions examined with the same methods used for the neutral ones.

The present investigation has shown that all the steroids from the main pathway of androgen biosynthesis are present in testicular tissue of *G. domesticus*, except 17 $\alpha$ -hydroxyprogesterone. The failure to detect 17 $\alpha$ -hydroxyprogesterone and the relatively low concentration of androstenedione could be due to a high desmolase and dehydrogenase activity in the gonad of this species. In vitro researches on progesterone metabolism by testicular tissue of the English sparrow seem to substantiate this hypothesis. In fact, 17 $\alpha$ -hydroxyprogesterone and androstenedione are found in the lowest concentration of any of the metabolites<sup>4</sup>.

The presence of 20 $\beta$ -hydroxypregn-4-en-3-one in rather high concentration could be interpreted in the light of the above-mentioned in vitro experiments of progesterone metabolism. It has been found that the sparrow testicular tissue is able to reduce 16% of the 20-ketone of progester-

one to both the 20 $\alpha$ - and 20 $\beta$ -isomers, while in the rat testis only 1% of the substrate is converted to the 20 $\beta$ -reduced compound<sup>4</sup>. According to FEVOLD and EIK-NES<sup>3</sup> the 2 reductases might be used to remove the excess of progesterone. In fact neither 20 $\alpha$ -hydroxypregn-4-en-3-one nor 20 $\beta$ -hydroxypregn-4-en-3-one show any androgenic activity in the sparrow beak assay<sup>5</sup>.

The absence of oestrogens in the cock testis, which have been detected in the testicular tissue of representatives of all other groups of vertebrates so far investigated, could be due to a low concentration below the sensitivity of our method.

If we compare these results with the ones obtained in reptiles<sup>6</sup>, we notice that several steroids, i.e. androsterone, dehydroepiandrosterone, oestradiol-17 $\beta$  and oestriol, which are present in high concentration in *Lacerta sicula* testes, are lacking in birds. Also androstenedione is present in very low concentration in birds, compared to the amount found in other vertebrates, including fishes<sup>6,7</sup>. The amount of testosterone is lower than that found in reptiles. The ratio of progesterone to testosterone is the same as that found in *Scyliorhinus stellaris* (elasmobranch)<sup>7</sup>, but the opposite of what has been found in reptiles, which approaches that of mammals<sup>8</sup>.

**Riassunto.** Nel tessuto testicolare di *Gallus domesticus* sono stati identificati i seguenti ormoni steroidi: pregnenolone (127  $\mu$ g/kg), progesterone (66,5  $\mu$ g/kg), androstenedione (2,4  $\mu$ g/kg), testosterone (35  $\mu$ g/kg), 20 $\beta$ -idrossipregn-4-ene-3-one (30,5  $\mu$ g/kg). Questi risultati dimostrano che il testicolo di pollo secerne gli steroidi della principale via biosintetica degli androgeni. La concentrazione relativamente alta di 20 $\beta$ -idrossipregn-4-ene-3-one viene discussa alla luce delle ricerche sul metabolismo in vitro del progesterone.

G. DELRIO, C. LUPO DI PRISCO and G. CHIEFFI

*Institute of Zoology and Comparative Anatomy,  
University of Camerino, Camerino (Macerata) and  
Zoological Station, Naples (Italy), 11th January 1967.*

<sup>1</sup> D. S. LAYNE, R. H. COMMON, W. A. MAW and R. M. FRAPS, *Nature* 181, 351 (1958).

<sup>2</sup> D. S. LAYNE, R. H. COMMON, W. A. MAW and R. M. FRAPS, *Proc. Soc. exp. Biol. Med.* 94, 528 (1957).

<sup>3</sup> H. R. FEVOLD and K. B. EIK-NES, *Gen. comp. Endocrinol.* 3, 335 (1963).

<sup>4</sup> O. V. DOMINGUEZ, H. F. ACEVEDO, R. A. HUSEBY and L. T. SAMUELS, *J. biol. Chem.* 235, 2608 (1960).

<sup>5</sup> H. R. FEVOLD and K. B. EIK-NES, *Gen. comp. Endocrinol.* 2, 506 (1962).

<sup>6</sup> C. LUPO DI PRISCO, G. CHIEFFI and G. DELRIO, *Experientia* 23, 73 (1967).

<sup>7</sup> G. CHIEFFI and C. LUPO, *Nature* 190, 169 (1961).

<sup>8</sup> This investigation was supported by a research grant No. HD 01477-07 from the National Institute of Child Health and Human Development, Public Health Service, USA, and by the Consiglio Nazionale delle Ricerche of Italy.