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FORMATION OF 5β-ANDROSTANES AS THE MAJOR METABOLITES OF TESTICULAR TISSUE OF HUMAN CHORIONIC GONADOTROPIN TREATED ANGUILLA ANGUILLA AT THE SILVER STAGE

by

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1. Introduction

The European eel differentiates sexually in European inland waters, but supposedly spawns in the vicinity of the Sargasso Sea (1). At the time of their catadromous migration they aquire a silvery appearance, but their gonads remain immature. No gonadal ripening has been observed in specimens hindered from reaching the sea, whatever their age and size.

Numerours experiments by which various gonadotropic hormone preparations have been used to induce gonadal ripening in the eel, have been performed (3-5, 8). Although considerable progress has been achieved by these attempts, no viable larvae were sofar reared (4). Only in Anguilla japonica treated with salmon pituitaries, the final stages of fertilization, hatching and survival of larvae were achieved (12). Administration of large doses of human chorionic gonadotropin (HCG) to male silver eels induces complete testicular ripening in part of the treated specimens after about 60 days (4).

Steroid production by ovarian tissue of Anguilla anguilla at the silver stage has been investigated by Colombo and Colombo Belvedere (6). Only the usual pattern of steroid production, found in other

species of teleost, could be identified. Testicular tissue of eels at the silver stage incubated with progesterone or androstenedione produced mainly 11β-hydrolyandrostenedione (7). In the present report we show that following HCG treatment testicular tissue of the eel produces a unique steroid pattern, which was not noted in another species of vertebrates.

2. Materials and Methods

[7α-^{3}H] androst-4-ene-3,17-dione (sp. act. 3 Ci/mmole, NEN,) and [7α-^{3}H] progesterone (sp. act. 18 Ci/mmole, NEN) were purified shortly before use by thin-layer chromatography. Non-radioactive steroids were obtained from Makor, Jerusalem, Reagents were of analytical grade. NADPH and other fine chemicals were products of Sigma Co.

Eels, Anguilla anguilla at the silver stage were caught in the Baltic Sea and transferred to the Experimental Fisheries Station at Ahrensburg and kept in closed sea-water aquaria of about 80 l capacity, in which 40 mm diameter plastic tubes served as shelters for the eels. In each container were 12 males. They were not fed for the duration of the experiment. Water temperature fluctuated at 19 to 21°C. Gonadal maturation was induced by weekly intramuscular injections of 500 IU HCG per animal, over a period of 4 weeks. This induced well developed testes, but no spermatic fluid could be extracted by stripping.

Following decapitation, testicular tissue was dissected out, put on ice and cut with a razor blade into cubes of about 1 mm^3. Portions of 1 g tissue minces were transferred to 25 ml Erlenmeyer flasks, at the bottom of which 3.8 µC of [^{3}H] androstenedione, or 4.5 µC of [^{3}H] progesterone, and 10 µg of the corresponding cold steroid were deposited (methanol - propylene glycol, 1:1). To this, 5 ml of an incubation medium prepared according to Arai (2) was added. The flasks were preincubated for 2 min at 30°C and the reaction started by the addition of NADPH, and gentle agitation of the Dubnoff incubator for 3 h.

At the end of the incubation, the reaction was stopped by the addition of 2 times 3 volumes of acetone, followed by 3 volumes of methanol. The organic solvents were evaporated under reduced

pressure and the aqueous residue extracted with methylene dichloride. To the dry lipid extract 1 mg portions of the following steroids were added; androstenedione, progesterone, testosterone and 11-keto-testosterone (11KT) as markers and standards of recovery. The extract was applied to a 20 g Celite column and eluted according to the method of Siiteri (9).

Authentic carrier steroids were added to each thin-layer chromatographic separation. Subsequently, carrier steroids were added to portions of the radioactive extracts after preliminary experiments had provided information as to the nature of products contained. Radiactivity was determined in toluene (using the PPO-POPOP system) scintillation fluid, using a Packard Tri-Carb counter.

3. Results and Discussion

The elution pattern of an incubation of HCG stimulated testicular tissue with [^{3}H]androstenedione is depicted in Fig. 1. Seven radioactive peaks are discernible; they do not coincide with the added cold steroids.

The compounds under these peaks were tentatively identified by comparing their mobilities and those of their derivatives with the appropriate standards on several chromatographic systems. The final identification by recrystallization to constant specific activity has not yet been accomplished.

Peak 1 = 5β-androstane-3,17-dione; Peak 2 = 3α-Hydroxy-5β-androstane-17-one; Peaks 3 and 4 = not yet identified for lack of appropriate standards, but oxidation with Cr_2O_3 yielded in both peaks 5β-androstane,3,11,17-trione. Peak 5 = a mixture of 11β-hydroxy-androst-4-ene-3,17-dione and of 3^{α}_{β}-hydroxy-5ß-androstane-11β-ol-17-one. Peaks 6 and 7 are minor metabolites and were as yet not identified.

Two additional incubations of HCG stimulated testicular tissue with labeled progesterone gave a very similar elution pattern of the metabolites produced.

Only a single report by Colombo et al. (7) on incubations of minced non-treated testicular tissue of Anguilla anguilla at the silver stage has been published. In it labeled progesterone as well as labeled androstenedione were mostly converted to 11β-hydroxy-androstenedione. We confirm here the production of this steroid, bit the main metabolites in HCG treated testicular tissue of the eel, with both androstenedione and progesterone as precursors, were 5β-reduced C_{19}-steroids, compound not yet identified in gonadal

tissue of teleosts. Ungar et al. (1977) reported on the identification of 3α-hydroxy-5β-pregnan-20-one as the main metabolite from ovarian tissue produced from progesterone by Heteropneustes fossilis. Recently Terada et al. (1980) identified from immature hamster testicular tissue several 5β-C_{21} and 5β-C_{19}-steroids.

The significance of the present report is that in the HCG treated eel steroid metabolism of C_{21} as well as C_{19}-steroid precursors is shifted into the production of 5β-C_{19}-steroids, a new type of steroid metabolism which has not yet been noted in any gonadal tissue of a vertebrate.

4. References

1) D'Ancona, U.: Symp. Zool. Soc. London 1: 61-75, 1960

2) Arai, R.; Tamaoki, N.: Gen. Comp. Endocr. 8: 305-313, 1967

3) Boetius, J.; Boetius, I.; Hemingsen, A.M.; Bruun, A.I.; Möller-Christensen, E.: Meddr. Danm. Fiskeri - og Havunders N.S., 3:183-198, 1962

4) Boetius, I.; Boetius, J.: Dana vol 1: 1-29, 1980

5) Bruun, A.F.; Hemmingsen, A.M.; Möller-Christensen, E.: Acta Endocrinol. 2:212-226, 1949

6) Colombo, L.; Colombo Belvedere, P.: Gen. Comp. Endocr. 28, 371-385, 1976

7) Colombo, L.; Pesavento, S.; Johnson, D.W.: Gen. Comp. Endocr. Suppl. 3, 245-253, 1972.

8) Fontaine, M.; Bertrand, E.; Lopez, E.; Callamand, O.: C. R. Acad. Sci. 259: 2907-10, 1964

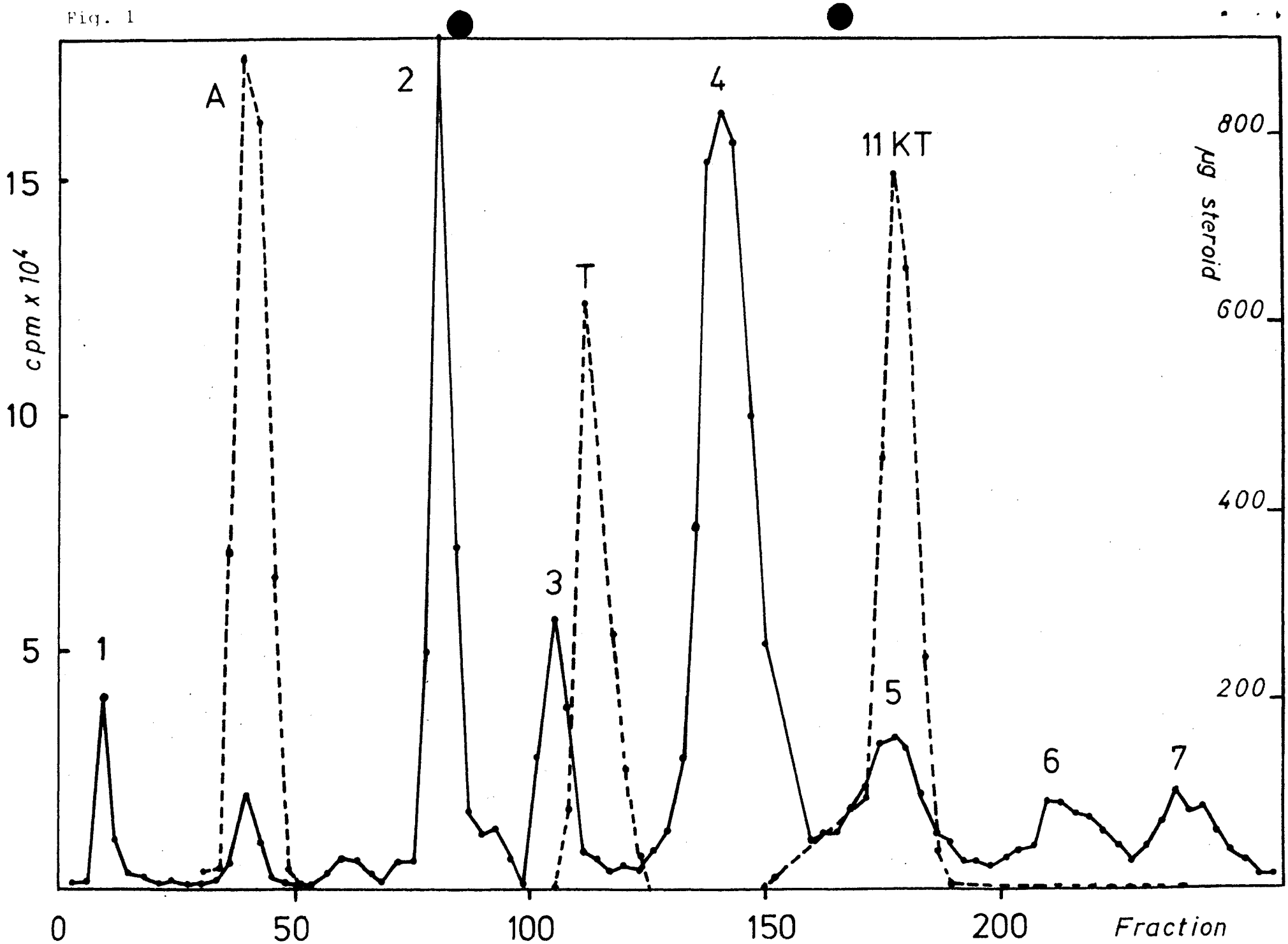
9) Siiteri, P.K.: Methods in Enzymology, vol. 36: 485-489, 1975

10) Terada, N., Sato, B.; Matsumoto, K.: Endocrinology 106: 1554-61, 1980.

11) Ungar, F.; Gunville, R.; Sundararaj, B.I.; Goswami, S.V.: Gen. Comp. Endocr. 31: 53-59, 1977.

12) Yamauchi, K.; Nakamura, M.; Takahashi, H.; Takano, K.: Nature 263: 412, 1976

Fig. 1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