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RADIOIMMUNOASSAY OF STEROID HORMONES PRODUCED BY EMBRYONIC CHICK GONADS DURING ORGAN CULTURE

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SUMMARY

Gonads from 7 1/2–18 days embryos were cultured on synthetic medium during 24 h. Radioimmunoassays after ether extraction of the media and of the homogenized organs, allowed quantitative determination of the amount and type of secreted steroids. Progesterone, dehydroepiandrosterone, estrone and estradiol-17β were assayed directly with specific antisera. Testosterone and dihydrotestosterone were previously separated by celite column chromatography. The sum of the steroids present in the explants and in their corresponding media were always higher than the hormone content of the gonads before culture, confirming a steroid production during the explantation. A sexual difference was observed: estrogens were mainly secreted by ovaries and very little estradiol-17β was produced by testes, which secrete more testosterone. At various developmental stages it seems that a 5α-reductase allowed, mainly in the embryonic testes, the formation of DHT.

INTRODUCTION

In previous works we studied the steroidogenesis from radioactive precursors, in chick embryonic gonads cultured *in vitro* [1, 2]. Male gonads were able to synthetize testosterone in increasing amount from pregnenolone, progesterone or dehydroepiandrosterone between 7 1/2 and 18 days of incubation and the estrogen synthetized from the same precursors and from sodium acetate by female gonads increased markedly during this period. In the present study the sex steroid production by explanted chick embryonic gonads was studied by specific radioimmunoassays.

MATERIALS AND METHODS

Gonads from white Leghorn chick embryos of 7 1/2–18 days incubation were explanted for 24 h on synthetic medium (Parker 199).

Methods for the analysis of culture media and homogenates of cultured tissues are shown in Fig. 1. 1. Separation in 5 aliquots of media and homogenized gonads. 2. Addition of 1000 c.p.m. of labeled tracers. 3. Extraction with ether (3 × 1.5 ml). 4. Celite column chromatography of one aliquot to separate testosterone and dihydrotestosterone (DHT). For chromatography, celite columns, packed in a glasswool-plugged disposable Pasteur pipettes were used (500 mg of celite in 0.25 ml of Formamide). Just before use the columns were washed with 1 × 5 ml heptane, the extracts (1 ml heptane) were transferred to the columns and these were eluted with: 4 ml heptane/benzene 98:2, 4 ml heptane/benzene 98:2 → DHT, 2 ml heptane/benzene 98:2, 3,8 ml benzene → testosterone. 5. Radioimmunoassay with specific steroid antisera for: Estrone and estradiol 6 CMO-BSA, testosterone 3 CMO-BSA, dihydrotestosterone 1α CH_2-CO-BSA, dehydroepiandrosterone 7 CMO-BSA (purchased by Pasteur Production), and antiprogesterone 11 NH-BSA (kindly given by J. P. Raynaud, Roussel–Uclaf).

The separation between free and bound steroids was performed by Charcoal/Dextran (2.5 g Norit A + 0.25 g Dextran T 70 in 1000 ml phosphate buffer 0,1 M pH 7.4). The mean value of the blank is very low, varying from 0–4 pg. The variability intra-assay

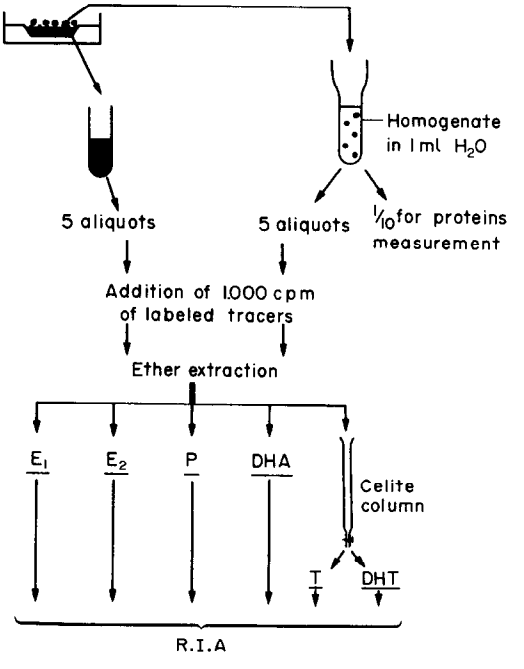

Fig. 1.

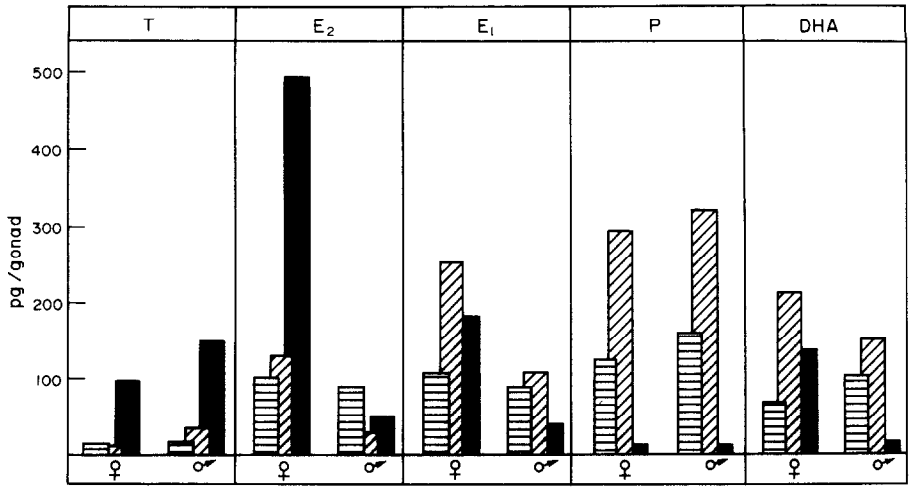

Fig. 2. Steroids content in gonads before ▤ and after ▨ culture, and in the culture medium ■ (left side at 10 days of incubation).

is from 2–11% and inter-assay from 6–13%. The amount of steroids assayed in the culture media is correlated to the number of explanted gonads. Between 6 and 10 different experiments were performed at each incubation stage. Unfortunately there are very important individual variations.

RESULTS

The steroid content of the freshly excised gonads, of the cultured organs, and of their corresponding media is shown in Fig. 2. Six steroids have been investigated: progesterone (P), dehydroepiandrosterone (DHA), testosterone (T), dihydrotestosterone (DHT), estrone (E_1) and estradiol (E_2). The relative amount of each steroid present in the cultured explants plus their corresponding media is always higher than the hormonal content in the gonads before culture, confirming a steroid production during the experiment.

Estradiol, testosterone and DHT are almost chiefly found in the culture media whereas progesterone, DHA and estrone are more or less retained in the gonads. Consequently all our results are expressed as the sum of the steroid content of the cultured tissue and its corresponding medium. The present paper only deals with the production of estrone, estradiol, testosterone and DHT by left chick embryonic gonads. The study of metabolic intermediates such as progesterone and DHA will be considered elsewhere. However all the results of each individual steroid are expressed as a percentage of the total hormonal production.

Testosterone and DHT production

As illustrated in Fig. 3, testosterone is produced by male and female gonads from 7 1/2 to 18 days. The quantitative difference between both sexes is statistically significant ($0.001 < P < 0.05$) except at day 18. The relative production of testosterone by male gonads increases to a peak concentration at day 10. The percentage of DHT production by the testes seems also to be more important at day 10 as well as at day 18 (one experiment only) than at day 7 1/2.

Estrone and estradiol-17β-production

As shown in Table 1 and Fig. 4, estrone and estradiol-17β ($E_1 + E_2$) are produced by the left gonads of both sexes. The relative production which is higher in ovaries than in testes ($p < 0.05$) at each stage of incubation remains nearly constant between 7 1/2 and

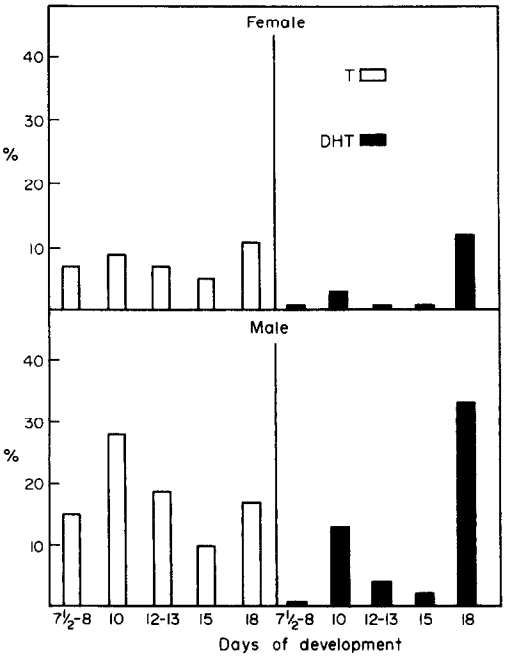

Fig. 3. Testosterone and DHT expressed as % of total production.

Table 1. Estrogen production ($E_1 + E_2$) in left gonads expressed as % of total steroids

% Of total production	Estrone + estradiol
7–8 j	♀ = 70% ♂ = 35%
10 j	♀ = 60% ♂ = 20%
12–13 j	♀ = 60% ♂ = 29%
15 j	♀ = 70% ♂ = 19%
18 j	♀ = 58% ♂ = 24%

18 days. In both sexes, estrone production is always more important than estradiol-17β and especially the male gonads do not secrete significant amounts of estradiol-17β (Fig. 4).

DISCUSSION

The results of the present investigation provide data on sex steroid production by chick embryonic gonads in culture and show a preferential relative production according to the sex. Male gonads synthesize testosterone and DHT with a peak concentration at 10 and at 18 days. Female gonads synthesize chiefly estrogens but also testosterone.

The present results concerning testosterone production are in agreement with those of Woods *et al.* [6, 7] obtained by immunohistochemistry in chick embryonic gonads and in plasma by radioimmunoassay.

The question arises if the relative production of testosterone by male gonads which increases to a peak concentration on day 10 may be correlated with the regression of male Müllerian ducts in the chick embryo. Contradictory results concerning testosterone action on the behaviour of Müllerian ducts have been obtained.

Recently, the regression of the Müllerian ducts cultured in contact with a suspension of testosterone microcrystals was observed [3]. A stimulating effect on the development of Müllerian ducts by both testosterone and dihydrotestosterone was also observed [4], suggesting that the testicular hormone responsible for the regression of the Müllerian ducts would not be an androgen. Moreover, the effects of exogenous testosterone as teratogenic were considered [5]. Since androgenic hormones are secreted by chick embryonic testes as shown in the present study an alternate explanation for the regression of male ducts in the chick embryo could be the intervention of androgens or of a protein induced by them.

Work is in progress in which the addition of precursors like pregnenolone or DHA allows a more precise study of the enzymatic activities of the embryonic gonads.

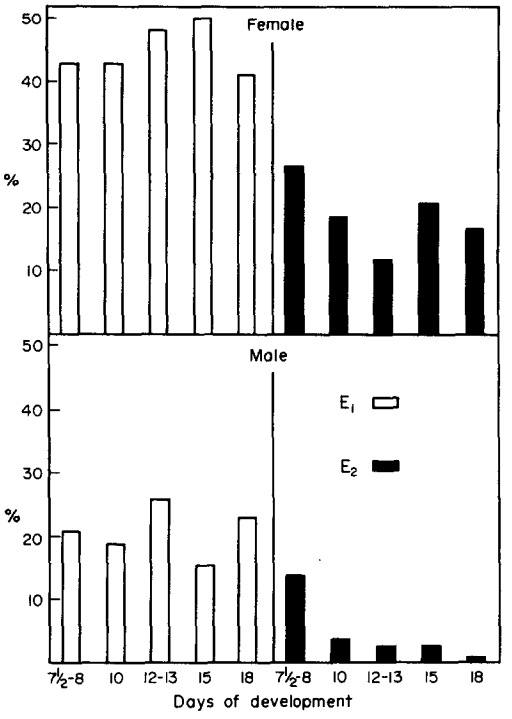

Fig. 4. Estrone and estradiol expressed as % of total production.

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DISCUSSION

Groenendijk. I was very interested hearing that you could demonstrate testosterone in the embryonic ovaries as well as in the embryonic testes. I wonder whether you can transfer your observations *in vitro* to the *in vivo* animal, as we all know that particularly the right ovary of the chick embryo is turning into a testis after a certain period of culture *in vitro*. I wonder if you have comparable observations on the ovaries *in vivo* at similar embryonic ages.

Guichard. I think that the organotypic culture *in vitro* is a good model to study the steroidogenesis in the embryonic gonads. Effectively, in the experiments with radioactive precursors, the culture allows the accumulation of the steroids produced, and enables us to quantify them.

To answer the second part of your question, I shall say that in our radioimmunoassays, we study systematically, at each stage of the development: the medium and the gonads after culture but also before explantation which gives an appreciation of the *in vivo* situation of the tissue.

Groenendijk. I am not altogether convinced, but that's a matter of opinion. My second question is this: if I understood you well, you linked testosterone production of the embryonic gonads to the Müllerian duct inhibition. I think it is a very controversial point that you brought up. Your conclusion was based on the observation that the testosterone production is increasing from the 11th to 18th day. Is this correct?

Guichard. No, that's not exactly true.

Groenendijk. I think one could argue the other way around by the fact that in my work it was demonstrated that the Müllerian duct inhibiting capacity of the embryonic testis is highest on the 6th day of incubation; afterwards it is declining gradually (*Anat. Anz.* **135** (1974) 43–46). Therefore I would say, if you find testosterone production increasing towards the end of the incubation, this is just an argument against the concept that testosterone is involved in Müllerian duct inhibition. Would you please comment on that?

Guichard. Effectively, we think that the relative production of testosterone by male gonads which reaches a maximum value at 10 days may be correlated with the regression of Müllerian ducts.

It is interesting to note that in preliminary results we have obtained in the right female gonad, an amount of testosterone which also reaches a maximum value at 10 days. This relative production of testosterone is significantly higher in right female gonad than in the left one and may be also correlated with the regression of Müllerian duct in this side, which appears between 9 and 13 days of development.

Groenendijk. But actually the main regression in the female occurs from the 9th day, particularly if you implant testicular tissue, and in the male the regression also takes place on the 9th day. Therefore it is rather late if you have an increase in testosterone from the 11th day. In my observations the 6th day old testis is the most potent in bringing about the Müllerian duct inhibition. Therefore I would say your observations can just as well be used as argument *against* the androgen influenced regression.