Δ^5 3β-Hydroxysteroid Dehydrogenase Activities in the Ovary of the Rice-Field Eel, *Monopterus albus* (Zuiew)[1]

It has been previously reported that the ovary of the protogynous hermaphrodite, *Monopterus albus*, is capable of producing both oestrogens and androgens in vitro (CHAN and PHILLIPS[2]). However, the site of steroid production has not been located. Since 3β-hydroxysteroid dehydrogenase (3 β-HSD) is fundamental in steroidogenesis (SAMUELS et al.[3]; SAMUELS and HELMREICH[4]), location of this enzyme would pin-point the steroidogenic tissue in the ovary before sex reversal from female to male.

Monthly samples of *Monopterus* ovaries were frozen on solid carbon dioxide, sectioned at 20 µm thickness in a Slee cryostat (London) maintained at −20 °C and directly placed on cover-slips. The histochemical method is a modification of the technique after WATTENBERG[5]. The sections were incubated for 3 h at 37 °C in the following media:

	Test	Control
Sodium phosphate buffer pH 7.5	4.2 ml	5.0 ml
Nitro-blue tetrazolium (NBT) (1 mg/ml in distilled water)	2.0 ml	2.0 ml
Nicotinamide adenosine dinucleotide (NAD) (6 mg/ml in distilled water)	1.0 ml	1.0 ml
Steroid substrate (DHEA) (1 mg/ml in dimethyl-formamide)	0.8 ml	0 ml

After incubation, the sections were fixed in 10% formalin for 10 min, dehydrated through the usual series of ethanol, cleared in xylene and mounted in Canada Balsam or DPX. Some sections were not dehydrated and mounted directly using aquemount. The tissues were also investigated for 17β-hydroxysteroid dehydrogenase activities using 17β-oestradiol as the substrate.

The follicle in the *Monopterus* ovary, as in many fish species, consists of an oocyte surrounded by an outer layer of elongated thecal cells and an inner layer of cuboidal granulosa cells (Figure 1). Only ovaries from eels sampled in the breeding season (May to July) showed positive reactions for 3β-HSD activities and the reaction was confined to the granulosa cells of large maturing follicles (Figures 2 and 3); the small follicles and primary follicles showed no observable reaction. Positive reactions for 3β-HSD were also observed in some cells in the interstitium of some ovaries (Figure 2) but were not confined to fish in the breeding season. In most ovaries that showed a positive reaction in the granulosa cells, interstitial cell activities for the same enzyme were also observed. No positive reaction for 17β-HSD activities was observed in any of the ovarian samples investigated.

3β-HSD activities were found in the granulosa cells, as in the ovary of *Poecilia reticulata* (LAMBERT[6,7]) and

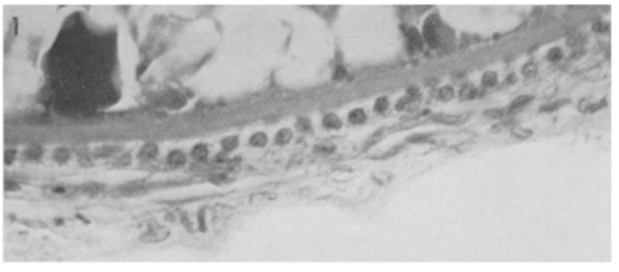

Fig. 1. Part of a large follicle in the *Monopterus* ovary, showing the follicular epithelium with the outer layer of elongated thecal cells and the inner layer of cuboidal granulosa cells. 8 µm, haematoxylin and eosin. ×320.

[1] This work was supported by grants from the Nuffield Foundation, London, and from the University of Hong Kong.

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3β-hydroxysteroid dehydrogenase activities in the ovary of *Monopterus albus*

Month	Total No. of fish	Reaction in the granulosa cells[a]	Reaction in the interstitial cells
January	2	− (2)	++ (2)
February	3	− (3)	++ (3)
March	———	———	———
April	9	− (8) + (1)	− (9)
May	6	− (3) ++ (3)	− (2) ++ (4)
June	7	− (4) ++ (3)	− (4) ++ (3)
July	6	− (5) + (1)	− (3) ++ (3)
August	5	− (5)	− (5)
September	———	—	———
October	3	− (3)	− (3)
November	4	− (4)	++ (4)
December	———	———	———

( ), No. of fish that showed the reaction; ———, No investigation undertaken; −, negative reaction. [a] Intensity of positive reaction graded subjectively as + or ++.

Acanthobrama terraesanctae (YARON[8]) and not in the thecal cells, as reported by BARA[9] in the ovary of *Scomber scomber*. In *Monopterus albus*, the presence of *3β*-HSD activities in the granulosa cells of the ovarian follicles albeit only in the active period of the sexual cycle provides strong evidence that these cells are capable of producing sex steroids. This, together with the previous findings that oestrogens were produced by the *Monopterus* ovary in vitro (CHAN and PHILLIPS[2]), indicates that the granulosa cells are the sites of oestrogen production. Negative reactions for 3β-HSD in ovaries during other parts of the sexual cycle and for 17β-HSD throughout the whole year may possibly be due to the limitation in the sensitivity of the histochemical technique employed. The demonstration of 3β-HSD in the breeding season is perhaps associated with the increase in the production of oestrogen, required for vitellogenesis.

3β-HSD activities were also found in the interstitial cells of some *Monopterus* ovaries where these cells were present. Interstitial cell activities for 3β-HSD in other teleostean ovaries seem to have only been reported in *Cymatogaster aggregata* (WEIBE[10]). Whether these cells secrete oestrogens or androgens remains unknown. So far no work has been done on the steroid enzyme histochemistry in any fish that undergoes natural sex reversal. The present study indicated that the *Monopterus* ovary possesses the enzymes essential for steroidogenesis. The significance of this is at present under investigation.

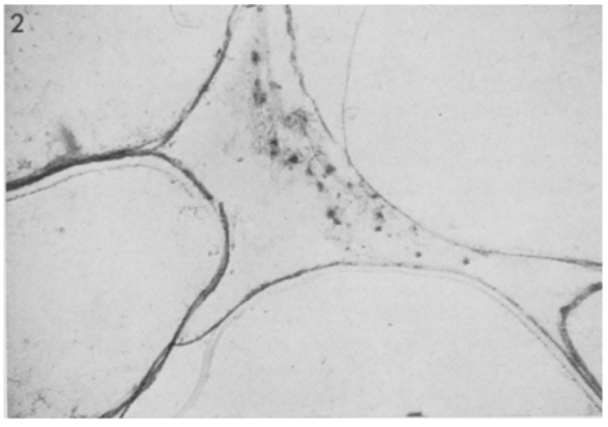

Fig. 2. Part of the ovary showing 3β-hydroxysteroid dehydrogenase activities in the granulosa cells and in the interstitial cells. May sample. 20 μm. ×28.

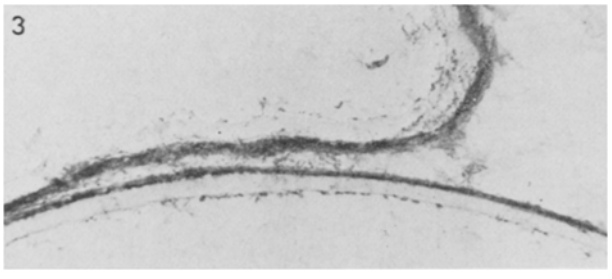

Fig. 3. Detail of the follicular epithelium of a mature follicle showing 3β-hydroxysteroid dehydrogenase activities in the granulosa cells. 20 μm. ×80.

Résumé. L'histochimie des enzymes de l'ovaire de *Monopterus* révèle la présence d'activités deshydrogénase hydroxystéroïde-3β dans les cellules granuleuses des grands follicules pendant la saison de la reproduction. Cela indique que les cellules granuleuses de ces follicules peuvent produire des steroïdes, peut-être des oestrogènes. Des cellules interstitielles de l'ovaire de *Monopterus* présentent aussi des réactions de deshydrogénase hydroxysteroïde-3β positives, mais il est impossible de décider si ces cellules produisent des androgènes ou des oestrogènes.

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15 August 1973.

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PRO EXPERIMENTIS

A New Colorimetric Method for the Assay of the Serum Glyoxalase System

The glyoxalase system consists of 2 enzymes: glyoxalase I (*S*-lactoylglutathione methylglyoxal-lyase E.C. 4.4.1.5) which produces lactoylglutathione, and glyoxalase II (*S*-2-hydroxyacylglutathione hydrolase E.C. 3.1.2.6) which splits off free glutathione. The reactions catalyzed by the 2 enzymes can be written:

$$CH_3COCHOHSG \underset{\text{(I)}}{\rightleftharpoons} CH_3CHOHCOSG$$

$$CH_3CHOHCOSG + H_2O \underset{\text{(II)}}{\rightleftharpoons} CH_3CHOHCOOH + GSH$$

Sum:

$$CH_3COCHOHSG + H_2O \rightleftharpoons CH_3CHOHCOOH + GSH$$

The first substrate is formed spontaneously from methylglyoxal and glutathione (GSH) in a reaction with a K_s value of 2×10^{-3} *M*. The reaction (I), catalyzed by glyoxalase I, has a K_m value of 0.19×10^{-3} *M* for the hemimercaptal, as shown with porcine erythrocytes enzyme by MANNERVIK et al.[1]. The importance of the glyoxalate system in cell growth regulation has been discussed extensively by FRENCH and FREEDLANDER[2] and by EYGUD and SZENT-GYORGYI[3]. The assays, used and described by RACKER[4], include a spectrophotometric technique at 240 nm, a manometric method and a colorimetric method based on hydroxamic acid formation.

Using the –SH group reagent of ELLMAN[5] we have developed a continuous spectrophotometric technique the rate of change of absorbance (Δ_2A/min) again recorded. The difference (Δ_2A/min − Δ_1A/min = ΔA/min) re-

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