Reprod. Nutr. Dévelop., 1982, **22** (2), 413-425.

Steroidogenesis in the gonads of rainbow trout fry *(Salmo gairdneri)* before and after the onset of gonadal sex differentiation

R. van den HURK, J. G. D. LAMBERT, J. PEUTE

Zoological Laboratory, Transitorium III, Section Comparative Endocrinology, Padualaan 8, Utrecht, The Netherlands.

Summary. Gonadal homogenates of rainbow trout from D(ay) 50, D100 and D200 after fertilization have been incubated *in vitro* in the presence of dehydroepiandrosterone-^{3}H to demonstrate 3β-hydroxysteroid dehydrogenase (3β-HSD) and Δ5,4-isomerase activity, of pregnenolone-^{3}H to assess the synthesis of « progestins » and of androstenedione-^{3}H to determine oestrogen synthesis in the ovaries and the formation of androgens in the testes. Histologically indifferent gonads from D50 contained 3β-HSD, Δ5,4-isomerase and 17α-hydroxylase, indicating a capacity to synthesize progestins. Ovaries from D100 possessed the same enzymes as D50 gonads ; those from D200 had, in addition, 17β-HSD and aromatase, indicative of oestrogen synthesis. Unlike the D50 gonads, the D100 testes also contained 17α, 20-desmolase and 11β-hydroxylase, showing a capacity to synthesize androgens. 17β-HSD was only demonstrated in D200 testes. The possible role of androgens and progestins in the regulation of gonadal sex differentiation in rainbow trout has been discussed.

Ultrastructural and enzymecytochemical investigations have demonstrated stroma (Leydig) cells as sources of steroidogenesis in rainbow trout testis from D100 onwards. The appearance of big mitochondria with many tubular cristae in those cells synchronized with the appearance of 11β-hydroxylase activity. Obvious steroidogenic sites could not be demonstrated in younger gonads and developing ovaries.

Introduction.

Based on the results from experiments with *Oryzias latipes* and *Carassius auratus,* which were treated with several steroids, Yamamoto (1969) reached the conclusion that sex steroids were natural sex-inducers, androgens acting as andro-inducers and oestrogens as gyno-inducers. This hypothesis, however, was not supported by the EM studies of Satoh (1974) who reported the sex differentiation of germ cells and somatic cells in the gonads of *Oryzias latipes* before the differentiation of steroid-secreting cells. Furthermore, the transplantation of the gonadal tissue of this species into the anterior eye-chamber of adults showed that the genetic male gonad differentiated into a testis regardless of the host's sexuality (Satoh, 1973). Muller (1969) and Hackmann

(1974), moreover, showed the paradoxal effects on gonadal sex differentiation of administering methyltestosterone to several *Cichlid* species.

Methyltestosterone administered to rainbow trout fry from hatching on results in gonadal sterilization (van den Hurk and Slof, 1981). When methyltestosterone treatment is started at the onset of gonadal sex differentiation, the androgen has a masculinizing effect on the gonads (Johnstone *et al.*, 1978 ; Okada *et al.*, 1979 ; van den Hurk and Slof, 1981). A feminizing effect is observed when the fry are treated with oestradiol-17 β (*) (Johnstone *et al.*, 1978) or progesterone (van den Hurk and Slof, 1981). Exogenous steroids thus effect gonadal sex differentiation in rainbow trout. Whether endogenous steroids regulage gonadal sex differentiation, however, is unclear. Data on the steroidogenic potency of gonadal tissue at the onset of gonadal sex differentiation would provide additional information about the possible role of endogenous steroids in this process. So far, there are no publications on steroid metabolism in juvenile fish before and just after gonadal sex differentiation.

The present study presents a biochemical, enzymecytochemical and ultrastructural study of the gonads of rainbow trout fry. *In vitro* incubations of homogenized gonads with ^{3}H-labeled precursor steroids were carried out to determine the steroidogenic potency of the gonads before and after the histologically visible start of gonadal sex differentiation. The steroid precursors used were : DHA-^{3}H to demonstrate 3β-HSD and Δ5,4-isomerase activities, P_5-^{3}H to assess the synthesis of « progestins » and Adione-^{3}H to determine oestrogen synthesis in ovaries and the formation of androgens in testes. Enzymecytochemical and ultrastructural studies were carried out to locate steroidogenic activity in the gonads.

Material and methods.

Animals. — Rainbow trout *(Salmo gairdneri)* fry were reared in aquaria at 11.5 ± 0.5 °C under a constant light regime of 12HL : 12HD. Hatching occurred at D29 after fertilization. First feeding started at D50 using commercial food (Trouvit). After anaesthesia with MS 222, the gonads were removed and then

---

(*) The following abbreviations and trivial names are used in this paper :

P_5	: (pregnenolone)	3β-hydroxy-5-pregnene-20-one
17α-P_5	: (17α-hydroxypregnenolone)	3β, 17α-dihydroxy-5-pregnene-17-one
DHA	: (dehydroepiandrosterone)	3β-hydroxy-5-androstene-17-one
P_4	: (progesterone)	4-pregnene-3,20-dione
17α-P_4	: (17α-hydroxyprogesterone)	17α-hydroxy-4-pregnene-3,20-dione
Adione	: (androstenedione)	4-androstene-3,17-dione
T	: (testosterone)	17β-hydroxy-4-androstene-3-one
11β-T	: (11β-hydroxytestosterone)	11β, 17β-dihydroxy-4-androstene-3-one
11-keto-T	: (11-ketotestosterone)	17β-hydroxy-4-androstene-3,11-dione
11β-Adione	: (11β-hydroxyandrostenedione)	11β-hydroxy-4-androstene-3,17-dione
11-keto-Adione	: (11-ketoandrostenedione)	4-androstene-3,11,17-trione
E_1	: (oestrone)	3-hydroxy-1,3,5,(10)-oestratriene-17-one
E_2	: (oestradiol-17β)	3,17β-dihydroxy-1,3,5,(19)-oestratriene

prepared for *in vitro* incubations or for histological, enzymecytochemical and ultrastructural studies.

Radioactive steroids. — Pregnenolone-7α-^{3}H (spec. act. : 666 GBq/mmol), androstenedione-7α-^{3}H (spec. act. : 303,4 GBq/mmol) and dehydroepiandrosterone-7α-^{3}H (spec. act. : 536.5 GBq/mmol) from the Radiochemical Centre, Amersham (UK) were purified by thin-layer chromatography (TLC) before use.

Chromatography. — TLC was carried out on precoated plates (10 × 20 cm) with silica gel F 254 (Merck AG) in saturated tanks, with the following systems : (I) toluene-cyclohexane (1 : 1) ; (II) dichloromethane-methanol (97 : 3) ; (III) Diisopropylether-chloroform-hexane (7 : 2 : 1) ; (IV) benzene-ethylacetate (3 : 1) ; (V) toluene-ethylacetate (3 : 1). After developing the plates, the 3-keto-Δ^4-steroids were located by UV-absorption, whereas the other steroids were detected by spraying with primuline (Wright, 1971).

Microchemical reactions. — Formylation was performed by dissolving the dry steroid in 0.5 ml formic acid 98 p. 100 for 2 h at room temperature. Acetylation was carried out overnight with 0.5 ml pyridine-acetic anhydride (1 : 1) at room temperature.

Measurements of radioactivity. — Samples were assayed using a Searle Analytic 92 scintillation counter with Xylofluor as a scintillator (Lumac). Radioactive areas on the TLC plates were located by means of a Berthold thin-layer radiochromatogram scanner.

Recrystallization. — Recrystallization to constant specific activity was carried out using aqueous methanol as a solvent. The crystals were quantified with a Mettler ME 30 balance (Axelrod *et al.,* 1965).

Incubation procedure. — A detailed description of the biochemical method has been published (Lambert and Pot, 1975 ; Lambert and van Bohemen, 1979). Gonads of D50, D100 and D200 fish were weighed and homogenized at 0 °C in 0.1 M phosphate buffer (pH 7.4) containing 0.25 M sucrose. The composition of the incubation mixtures are shown in table 1. Incubations were carried out in an air atmosphere under continuous shaking. After 3 h the enzyme reactions were terminated by adding dichloromethane.

Extraction. — Unlabeled carrier steroids (± 100 µg of each) were added to the incubation mixture before extraction with dichloromethane (3 × 10 ml). The combined dichloromethane extracts were evaporated *in vacuo* and the residue was subjected to TLC in system I to separate apolar compounds (triglycerides) from steroids. In this system the steroids remain localized on the base line, so it is possible to use the same plate for a first separation of the steroids.

Incubations with DHA-^{3}H. — Unlabeled DHA and Adione were added as carriers before extraction with dichloromethane. Both carriers were separated by TLC with system II. The ^{3}H-labelled Adione area was eluted and the ^{3}H-compound appeared to resist formylation. Finally, the ^{3}H-compound corresponding

TABLE 1

Homogenate and composition of the incubation mixtures.

	D50 indifferent gonads			D100 ovaries/testes			D200 ovaries/testes	
Homogenate (mg tissue/ml sucrose buffer)	2.5	2.5	2.5	5.2	5.2	5.2	100	100
Incubation mixture ^{3}H-precursor	P_5 (37 kBq)	DHA (37 kBq)	Adione (70 kBq)	P_5 37 kBq)	DHA (37 kBp)	Adione (70 kBq)	DHA (37 kBq)	Adione (70 kBq)
Propylene glycol (ml)	0.025	0.025	0.025	0.025	0.025	0.025	0.3	0.3
Cofactor (2mM each)	NAD/NADPH	NAD	NAD/NADPH	NAD/NADPH	NAD	NAD/NADPH	NAD	NAD/NADPH
Phosphate buffer (ml)	0.2	0.2	0.2	0.15	0.15	0.15	1.2	1.2
Homogenate (ml)	0.2	0.2	0.2	0.25	0.25	0.25	0.5	0.5

with the Adione carrier was purified by recrystallization to a constant specific activity.

Incubations with P_5 ^3H. — After incubation the following steroids were added as carriers : P_5, P_4, 17α-P_5, 17α-P_4, DHA, Adione, 11β-Adione and T. The first separation of steroids was carried out by TLC in system IV (3 ×). The areas corresponding to the added carriers were eluted, *e.g.* A : P_4 ; B : P_5, DHA and Adione ; C : 17α-P_5, 17α-P_4 and T ; D : 11β-Adione. TLC in system II of fraction B affected a separation of a combined P_5 and DHA fraction and an Adione fraction. After TLC in system V it was possible to separate DHA from P_5. The carriers in fraction C were separated with TLC in system II. The ^3H-labeled steroid fractions were further purified by recrystallization to a constant specific activity.

Incubation with Adione-^3H.

A. *Testes and D50 gonads.* — The carriers added were : Adione, T, 11-keto-Adione, 11β-Adione, 11-keto-T and 11β-T. The first separation was carried out with TLC in system IV. The areas corresponding to the added carriers were eluted, *e.g.* A : Adione ; B : T and 11-keto-Adione ; C : 11β-Adione and D : 11β-T and 11-keto-T. All these fractions were subjected to TLC in system II resulting in a purification of the fractions containing Adione and 11β-Adione and in a separation of T and 11-keto-Adione of fraction B and of 11β-T and 11-keto-T of fraction D. The ^3H-labeled steroid fractions were further purified by recrystallization to a constant specific activity.

B. *Ovaries.* — Before extraction the following steroids were added as carriers : Adione, T, E_1 and E_2. The first separation, carried out with TLC in system III, effected a separation of the four added carriers. After elution of the areas containing ^3H-compounds that corresponded to the added carriers, the separated ^3H-compounds were consecutively subjected to TLC in system II. Finally, the ^3H-compounds corresponding to the acetates of T, E_1 and E_2 were purified by recrystallization to a constant specific activity.

Histology. — Before using gonads from D100 and D200 for *in vitro* studies, they were quickly examined under a microscope to establish the sex. Besides 5 gonads of both sexes, 10 gonads from D50 animals were fixed in Bouin-Hollande to determine gonadal differentiation. The gonads were embedded in paraffin and cut into 5-μm sections which were then stained with haemalum-eosin.

Enzymecytochemistry. — Gonads were removed from 10 fish on D50, D75, D100, D150, D200 and D350 each, subsequently frozen with CO_2 and cut at − 20 °C into 10-μm and 18-μm sections on a cryostat microtome. 3β-HSD and glucose-6-phosphate dehydrogenase (G6PD) were demonstrated according to the method of van den Hurk (1973).

Electron microscopy. — Gonads were removed from 5 fish on D50, D75, D100, D200 and D350 each for ultrastructural investigation. Whole gonads or parts of gonads were fixed according to Peute *et al.* (1976). The tissues were dehydrated in acetone and embedded in araldite or in graded alcohol and

propyleneoxide, followed by embedding in Epon. The sections were contrasted with uranylacetate in 70 p. 100 methanol and lead citrate and examined with a Zeiss EM-9 electron microscope.

Results.

Incubations. — The qualitative results of the gonadal incubation experiments with ^{3}H-pregnenolone, ^{3}H-androstenedione and ^{3}H-dehydroepiandrosterone as precursors at different days of gonadal development are summarized in table 2. It

TABLE 2

Steroids identified form in vitro *incubations of homogenized gonads with ^{3}H-labeled precursor steroids.*

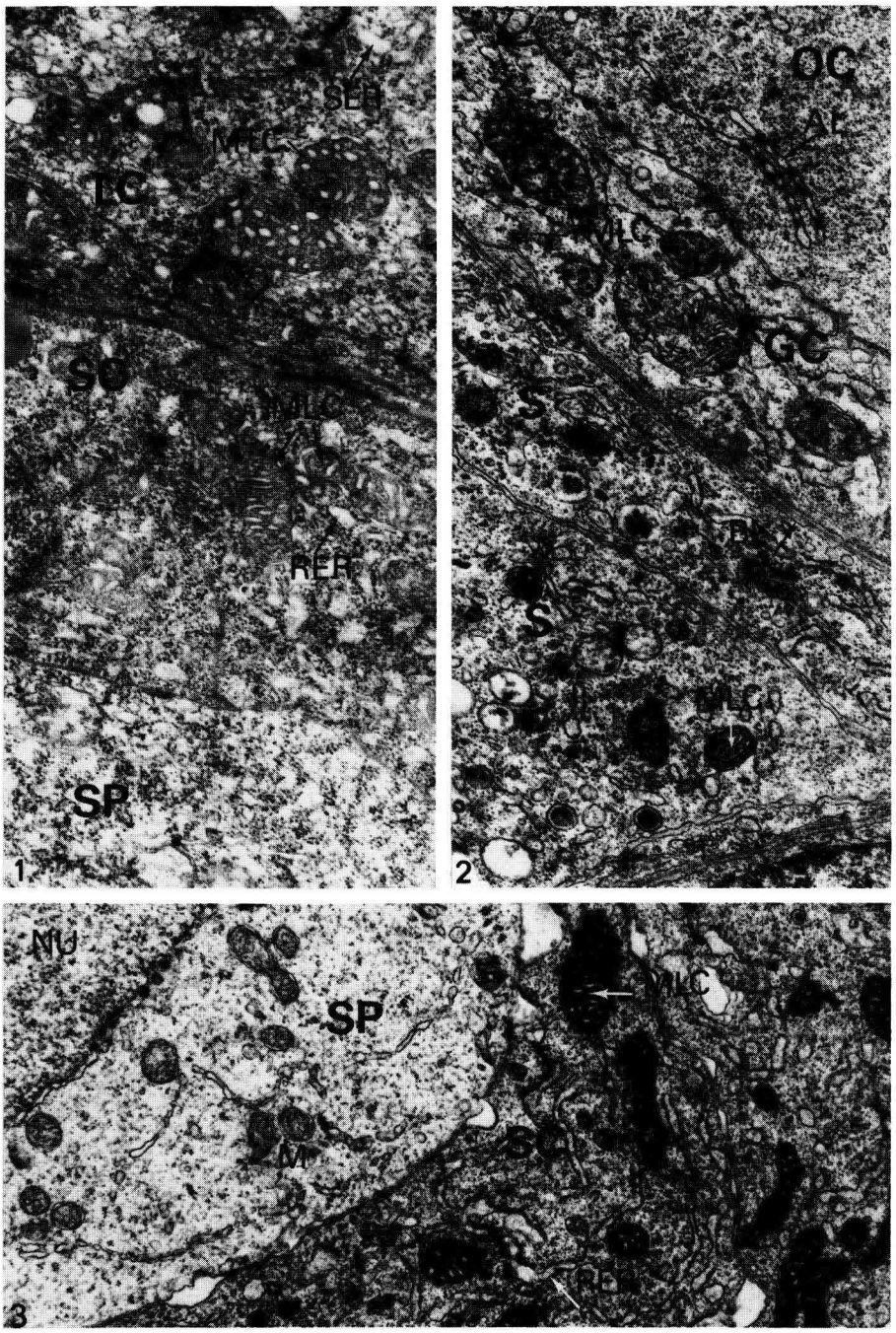
^{3}H-Substrate	Day	Gonad	Identified steroids
P_5	50	indifferent	P_5 ; P_4 ; 17αP_5
(NAD, NADPH)	100	testis	P_5 ; P_4 ; 17αP_5 ; 17αP_4 ; Adione ; 11β-Adione
	100	ovary	P_5 ; P_4 ; 17αP_5 ; 17αP_4
Adione	50	indifferent	Adione
(NAD, NADPH)	100	testis	Adione ; 11β-Adione
	200	testis	Adione ; 11β-Adione ; T
	100	ovary	Adione
	200	ovary	Adione ; T ; E_1 ; E_2
DHA	50	indifferent	DHA ; Adione
(NAD)	100	testis	DHA ; Adione
	200	testis	DHA ; Adione
	100	ovary	DHA ; Adione
	200	ovary	DHA ; Adione

appears that indifferent gonads at D50 already contained some enzymes involved in steroid synthesis ; 3β-hydroxysteroid dehydrogenase and 17α-hydroxylase were demonstrated. Contrary to the ovary at D100, the testis at D100 could synthesize androgens (Adione and 11β-Adione). The ovary at D200, but not at D100, appeared to contain the aromatase enzymes for synthesizing oestrogens.

FIG. 1. — *Ultrastructure of Leydig cells* (LC) *and Sertoli cells* (SC) *at D150.* MTC = mitochondrion with tubular cristae ; MLC = mitochondrion with lamellar cristae ; SP = spermatogonium. × 17 000.

FIG. 2. — *Ultrastructure of stroma cells* (S) *and granulosa cells* (GC) *at D150.* OC = oocyte ; BL = basal lamina ; AL = annulate lamellae. × 17 000.

FIG. 3. — *Part of a testis from D75.* Small mitochondria (M) in a spermatogonium (SP) and large mitochondria with lamellar cristae (MLC) in Sertoli cells (SC). NU = nucleus. × 17 000.


SER
MLC
TLC
SG
RER
SP
1
OC
AF
MLC
GC
S
SG
MLC
2
NU
SP
MLC
M
RER
3

Histology. — The D50 gonads were indifferent structures corresponding to a previous description (van den Hurk and Slof, 1981). Until D350, the testes were massive structures containing primary spermatogonia surrounded by Sertoli cells, blood capillaries, fibroblasts, connective tissue and nerve fibres. The ovaries were built up from lamellae that contained oogonia surrounded by granulosa cells, previtellogenic follicles in different stages of development and interstitial tissue. The interstitial tissue mainly consisted of fibroblasts, connective tissue and blood capillaries.

Ultrastructure. — Obivous steroidogenic cells, characterized by large amounts of big oval to elongated mitochondria with tubular cristae and SER, were only observed in the stroma of the testes from D100 onwards (fig. 1). Some of these stroma (Leydig) cells were innervated since nerve fibres showed synaptic contacts with these cells. Indifferent gonads, D75 testes and developing ovaries had stroma cells with predominant RER and rather small mitochondria with mainly lamellar cristae (fig. 2).

Granulosa cells, Sertoli cells and their homologues in indifferent gonads had RER and mitochondria with lamellar cristae besides many free ribosomes (figs. 1, 2).

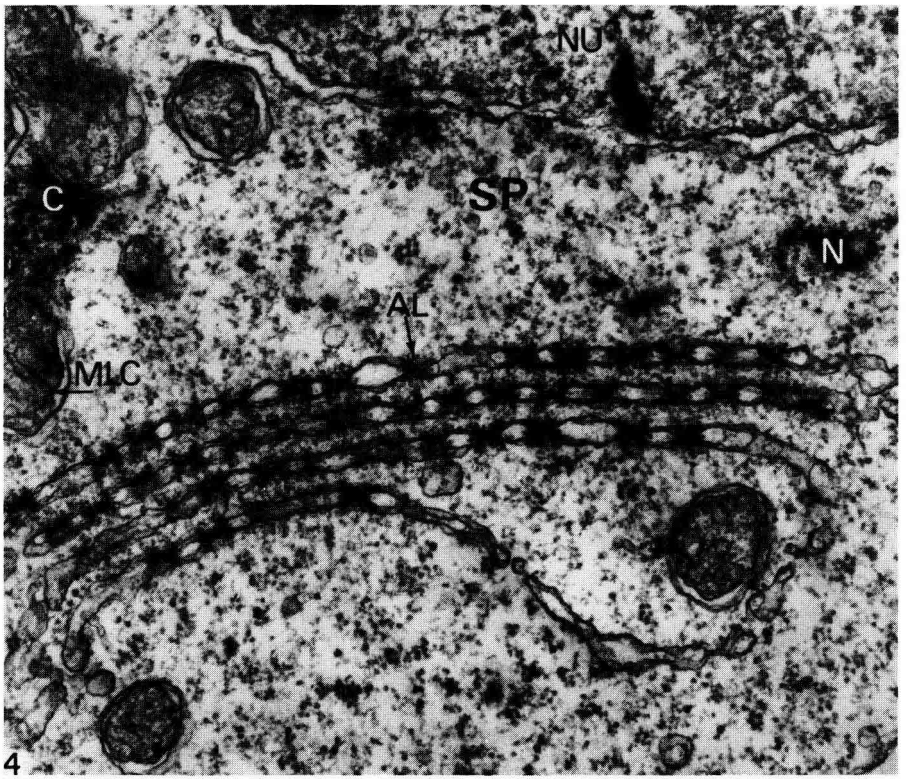
Primordial germ cells of D50 animals were empty in appearance. A few vesicular structures and small mitochondria without clear cristae characterized the undeveloped nature of their cytoplasm. From D100 until D350, the spermatogonia, oogonia and young oocytes had a well-developed, mainly smooth RER, many free ribosomes and rather small mitochondria with lamellar to tubular cristae (figs. 3, 4). Oocytes with microvilli at their cell membrane contained mitochondria with lamellar cristae and both RER and SER. Young germ cells furthermore contained « ciment » and « nuage » (fig. 4), as described by Clerot (1968), annulate lamellae as described by Kessel (1968) and well-developed Golgi cisternae.

Histochemistry. — Both 3β-HSD and G6PD activity have been demonstrated in testicular stroma cells from D100 onwards (fig. 5). Weak and moderate G6PD activities were visualized in the granulosa and stroma cells, respectively, of D350 ovaries (fig. 6). 3β-HSD activity, however, was absent at the latter sites.

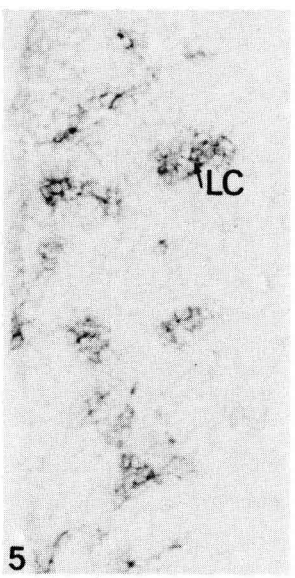
FIG. 4. — *Part of a spermatogonium* (SP) *from D100 with annulate lamella* (AL), « *nuage* » (N) *and* « *ciment* » (C). × 55 000.

FIG. 5. — *3β-HSD activity in testicular Leydig cells* (LC) *from D100.* × 560.

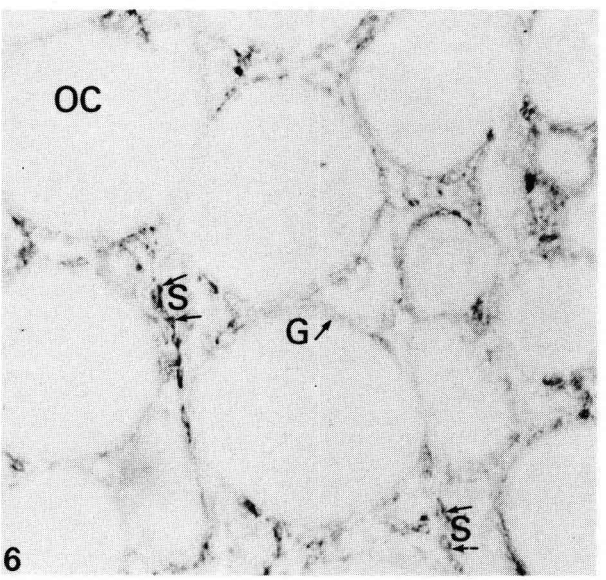
FIG. 6. — *G6PD activity in stroma cells* (S) *and granulosa* (G) *of ovaries from D350.* OC = oocyte. × 350.



4



5



6

Discussion.

There are no publications about steroidogenesis in the gonads of juvenile fish at the onset of gonadol sex differentiation. The present paper describes an *in vitro* study to demonstrate steroid bioconversions in the gonads of rainbow trout fry just before and after the time of gonadal sex differentiation. Contrary to D50 indifferent gonads, young testes from D100 onwards contained enzyme systems for synthesizing the androgens, Adione and 11 β-Adione, from pregnenolone as a precursor. Consequently, the capacity of androgen synthesis started in the testes between D50 and D100. It is unclear whether this development caused or resulted from testicular development. The present data demonstrating the capacity of the testes to synthesize androgens shortly after the onset of gonadal sex differentiation are thus insufficient to prove the hypothesis of Yamamoto (1969) that in fish androgens regulate the development of indifferent gonads into testes. The observations of Johnstone *et al.* (1978), Okada *et al.* (1979) and van den Hurk and Slof (1981) that in rainbow trout exogenous androgens are able to influence gonadal sex dfferentiation in a male direction, support the role of androgens at the onset of testicular development in fish.

It was not possible to demonstrate the synthesis of androgens or œstrogens in indifferent gonads at D50 and in ovaries at D100. The enzymes, 17α, 20-desmolase, 17β-HSD and aromatase, thus were probably absent. These gonads, however, could synthesize P_4, 17α-P_5 or 17α-P_4. The present data may point to the role of « progestins » in the regulation of gonadal sex differentiation in rainbow trout. These findings are supported by previous results (van den Hurk and Slof, 1981) from which it appears that the administration of P_4 around the start of gonadal sex differentiation leads to feminization of the gonads. It is possible to conceive of the effect of « progestins » as a direct feminizing action on the gonads or as an indirect one, via its inhibiting effect on androgen formation. An inhibitory effect of « progestins » on androgen synthesis has been described by Gower (1975). Endogenous « progestins » thus might block androgen synthesis in trout fry gonads and thereby the possibility of their developing into testes. The absence of androgens in the gonads at the onset of gonadal sex differentiation might result in ovarian development. This hypothesis contradicts the opinion of Yamamoto (1969) that œstrogens act as gyno-inducers. It is not likely that œstrogens act as gyno-inducers in rainbow trout since œstrogen synthesis could not be demonstrated in D100 ovaries.

The demonstration in D200 ovaries of enzymes synthesizing T, E_1 and E_2, and in D200 testes of those synthesizing T besides Adione and 11β-Adione, indicates that steroidogenesis progresses with age. This advanced capacity for steroid synthesis may indicate that T and œstrogens function to develop various organ systems at later stages of gonadal development.

No obvious steroid-synthesizing structures could be demonstrated in indifferent D50 gonads. Ultrastructural and enzymecytochemical investigations have clearly shown testicular stroma (Leydig) cells to be sites of steroidogenesis from D100 onwards, *i.e.* after the establishment of gonadal sex. The observed

location of steroidogenesis in trout testis corresponds to previous results with mature (van den Hurk *et al.,* 1978) and immature (Oota and Yamamoto, 1966 ; Upadhyay, 1977) *Salmo gairdneri.* The appearance of steroidogenenic cells after the onset of gonadal sex differentiation corresponds to the findings of Satoh (1974) on *Oryzias latipes.* The appearance of large mitochondria with an extensive network of tubular cristae in the Leydig cells coincides with the appearance of 11β-hydroxylase in testes from D100 onwards. In mammalian adrenal tissue, 11β-hydroxylase is located in such mitochondria (Malamed, 1975). The presence of 11β-hydroxylase has also been demonstrated in the testis of adult *Salmo gairdneri* (Arai and Tamaoki, 1967 ; Kime, 1979) and *Poecilia latipinna* (van den Hurk and Lambert, 1977) that both contain large mitochondria with tubular cristae (van den Hurk *et al.,* 1974, 1978).

The present morphological findings do not convincingly point to steroid sources in developing ovaries. Enzymecytochemical findings in the developing ovaries more or less correspond to those obtained in the mature ovaries of female rainbow trout during the previtellogenic period since ovaries at that time have few stroma cells with a weak 3β-HSD and moderate G6PD activity (van den Hurk and Peute, 1979). Ultrastructural data, however, rather point to young germ cells since they contain SER and small mitochondria with tubular cristae. The presence of these features in developing cells does not necessarily point to a steroidogenic capacity. Anderson *et al.* (1970) previously suggested that the number and modifications of the cristae indicate the stage of mitochondriogenesis and the level of enzymatic activity in which that organelle is engaged during oogenesis and early embryogenesis. Mitochondria with tubular cristae and SER have also been observed in the developing par distalis cells of young rainbow trout (Peute, unpublished). The absence of large mitochondria with tubular cristae in indifferent gonads and developing ovaries might be due to the lack of enzyme systems involved in the synthesis of steroids with a functional group at the 11-position.

Communication présentée au colloque sur la « Sexualisation chez les Vertébrés inférieurs », Banyuls-sur-mer, France, avril 1981.

Reçu en mai 1981.
Accepté en septembre 1981.

Résumé. Des homogénats de gonades de truite arc-en-ciel de 50, 100 et 200 jours après la fécondation ont été incubés *in vitro* en présence soit de déhydroépiandrostérone-^{3}H pour mettre en évidence les activités 3β-hydroxy-stéroïde-déhydrogénase (3β-HSD) et Δ 5,4-isomerase, soit de prégnénolone-^{3}H pour étudier la synthèse de la progestérone et de ces dérivés ; enfin en présence d'androstènedione-^{3}H pour étudier la synthèse d'œstrogènes dans les ovaires et la formation d'androgènes dans les testicules. Les gonades de 50 jours qui, selon les critères histologiques sont indifférenciées, contiennent des activités 3β-HSD, Δ 5,4 isomérase et 17α-hydroxylase, ce qui suggère que ces gonades sont capables de synthétiser des progestagènes. Les ovaires de 100 jours contiennent les mêmes enzymes que les gonades de 50 jours ; ceux de 200 jours possèdent en plus une activité 17β-HSD et une activité aromatase indiquant une capacité de synthèse d'œstrogènes. Des testicules de 100 jours ont les mêmes enzymes que les gonades de 50 jours, mais ont en plus 17α,20-desmolase et 11β-hydroxylase pour la synthèse

respectivement de l'androstènedione et de la 11β-hydroxyandrostènedione. En plus de ces androgènes, la testostérone peut être synthétisée par le testicule de 200 jours à partir de l'androstènedione, indiquant la présence de 17β-HSD. La capacité de synthétiser *in vitro* la testostérone, l'œstrone et l'œstradiol-17β montre la présence de 17β-HSD et d'aromatase dans des ovaires de 200 jours. Le rôle possible d'androgènes et de progestagènes dans la régulation de la différenciation des gonades chez la truite arc-en-ciel est discuté.

Des études ultrastructurales et histoenzymatiques montrent dans les testicules de 100 jours et plus, la synthèse de stéroïdes dans des cellules du stroma. Dans ces cellules de grandes mitochondries à nombreuses crêtes tubulaires apparaissent en même temps que l'activité 11β-hydroxylase. La localisation de l'activité stéroïdogène dans les gonades indifférenciées et les ovaires n'a pas pu être clairement démontrée.

References

ANDERSON E., CONDON W., SHARP D., 1970. A study of oogenesis and early embryogenesis in the rabbit, *Oryctolagus cuniculus,* with special reference to the structural changes of mitochondria. *J. Morphol.,* **130,** 67-92.

ARAI R., TAMAOKI B., 1967. Biosynthesis of 11β-hydroxytestosterone and 11-keto-testosterone in the testes of rainbow trout. *Can. J. Biochem.,* **45,** 1191-1195.

AXELROD L. R., MATTHIJSSEN C., GOLDZIEHER J. W., PULLIAM J. E., 1965. Definitive identification of microquantities of radioactive steroids by recrystallization to constant specific activity. *Acta endocr. Copenh.,* **49** (suppl. 99), 7-60.

CLEROT J. C., 1968. Mise en évidence par cytochimie ultrastructurale de l'émission de protéines par le noyau d'auxocytes de *Batraciens. J. Microsc.,* **7,** 973-992.

GOWER D. B., 1975. Regulation of steroidogenesis, 127-147. In H. L. J. MANKIN. *Biochemistry of steroid hormones,* Blackwell Scient. Publ., Oxford, London.

HACKMANN E., 1974. Einfluss von Androgenen auf die Geschlechtsdifferenzierung verschiedener Cichliden (*Teleostei*). *Gen. comp. Endocrinol.,* **24,** 44-52.

HURK R. van den. 1973. The localization of steroidogenesis in the testis of oviparous and viviparous teleosts. *Proc. kon. ned. Akad. Wet.,* **C76,** 270-279.

HURK R. van den, LAMBERT J. G. D., 1977. Biosynthesis of 11β-hydroxy and 11-ketoandrogens in the testis of the black molly, *Poecilia latipinna. Comp. Biochem. Physiol.,* **56B,** 117-118.

HURK R. van den, MEEK J., PEUTE J., 1974. Ultrastructural study of the testis of the black molly (*Mollienisia latipinna*). II. Sertoli cells and Leydig cells. *Proc. kon. ned. Akad. Wet.,* **C77,** 470-476.

HURK R. van den, PEUTE J., 1979. Cyclic changes in the ovary of the rainbow trout, *Salmo gairdneri,* with special reference to sites of steroidogenesis. *Cell Tissue Res.,* **199,** 289-306.

HURK R. van den, SLOF G. A., 1981. A morphological and experimental study of gonadal sex differentiation in rainbow trout, *Salmo gairdneri. Cell Tissue Res.,* **218,** 487-498.

HURK R. van den, PEUTE J., VERMEIJ J. A. J., 1978. Morphological and enzyme-cytochemical aspects of the testis and vas deferens of the rainbow trout, *Salmo gairdneri. Cell Tissue Res.,* **186,** 309-325.

JOHNSTONE R., SIMPSON T. H., YOUNGSON A. F., 1978. Sex reversal in salmonid culture. *Aquaculture,* **13,** 115-134.

KESSEL R. G., 1968. Annulate lamellae. *J. ultrastruct. Res.,* **Suppl. 10,** 1-82.

KIME D. E., 1979. The effect of temperature on the testicular steroidogenic enzymes of the rainbow trout, *Salmo gairdneri. Gen. comp. Endocrinol.,* **39,** 290-296.

LAMBERT J. G. D., BOHEMEN Ch. G. van, 1979. Steroidogenesis in the ovary of the rainbow trout, *Salmo gairdneri,* during the reproductive cycle. *Proc. ind. nat. Sci. Acad.,* **B45,** 414-420.

LAMBERT J. G. D., POT M. G. E., 1975. Steroidogenesis in ovarian tissue of a viviparous teleost, the guppy *Poecilia reticulata. Comp. Biochem. Physiol.,* **50B,** 585-589.

MALAMED S., 1975. Ultrastructure of the mammalian adrenal cortex in relation to secretory function. In : S. R. GEIGER. *Handbook of physiology.* Vol. **VI** : *Adrenal gland,* 25-29. Am. Physiol. Soc. Washington.

MÜLLER R., 1969. Die Einwirkung von Sexualhormonen auf die Geslechtsdifferenzierung von *Hemihaplochromis multicolor* (Higendorf)-(*Cichlidae*). *Zool. Jb. Physiol.*, **74**, 519-562.

OKADA H., MATUMOTO H., YAMAZAKI F., 1979. Functional masculinization of genetic females in rainbow trout. *Bull. jap. Soc. Sci. Fish.*, **45**, 413-419.

OOTA I., YAMAMOTO K., 1966. Interstitial cells in the immature testes of the rainbow trout. *Annot zool. jap.*, **39**, 142-148.

PEUTE J., BRUYN M. G. A., SELDENRIJK R., OORDT, P. G. W. J. van, 1976. Cytophysiology and innervation of gonadotropic cells in the pituitary of the black molly (*Poecilia latipinna*). *Cell Tissue Res.*, **174**, 35-54.

SATOH N., 1973. Sex differentiation of the fry gonad transplanted into the anterior chamber of the adult eye in the teleost *Oryzias latipes*. *J. Embryol. exp. Morphol.*, **30**, 345-358.

SATOH N., 1974. An ultrastructural study of sex differentiation in the teleost *Oryzias latipes*. *J. Embryol. exp. Morphol.*, **32**, 192-215.

UPADHYAY S. N., 1977. *Morphologie des gonades immatures et étude expérimentale de l'induction de la gamétogenèse chez la truite arc-en-ciel juvénile (Salmo gairdneri).* Th. Ph. D. Univ. P. et M. Curie, Paris.

WRIGHT R. S., 1971. A reagent for the non-destructive location of steroids and some other lipophilic materials on silica gel thin-layer chromatograms. *J. Chromat.*, **59**, 220-221.

YAMAMOTO T., 1969. Sex differentiation, 117-175. In *Fish physiology,* Vol. III. Acad. Press, New York and London.