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The Skin of the Male African Catfish, *Clarias gariepinus:* A Source of Steroid Glucuronides

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Steroid metabolism in the skin of mature male African catfish, *Clarias gariepinus*, reared in the laboratory, was studied *in vitro* by tissue incubations with [^{3}H]pregnenolone, [^{3}H]dehydroepiandrosterone, [^{3}H]17α-hydroxyprogesterone, [^{3}H]androstenedione, [^{14}C]11β-hydroxyandrostenedione, and [^{3}H]testosterone as precursors. While pregnenolone was not converted to any other steroid, dehydroepiandrosterone was transformed mainly to 5-androstene-3β,17β-diol. The products of 17α-hydroxyprogesterone incubations were 5β-pregnane-3α,17α-diol-20-one, 5β-pregnane-3α,17α, 20β-triol, and 5β-pregnan-17α-ol-3,20-dione. The major steroids of androstenedione incubations were etiocholanolone, testosterone, and androsterone. Testosterone was converted mainly to etiocholanolone and androstenedione, and only small quantities of 11β-hydroxytestosterone, 11-ketotestosterone, and 11-ketoandrostenedione were the metabolites found in 11β-hydroxyandrostenedione incubation. These results demonstrated the presence of the enzymes 5α- and 5β-reductases and 3α-, 11β-, 17β-, and 20β-hydroxysteroid dehydrogenases in the skin. From enzymehistochemical results it appeared that the steroid conversions take place in the epithelial cells. Moreover, the presence of UDP–glucose dehydrogenase, an enzyme involved in the synthesis of glucuronic acid, in these cells indicates the possibility of steroid glucuronide formation. Indeed significant amounts of water-soluble steroid conjugates, particularly 5β-dihydrotestosterone– and testosterone–glucuronide, were found in the incubations with androstenedione and testosterone, indicating the presence of the UDP–glucuronosyl transferase in the catfish skin. In the light of these results, a role of the skin of African catfish in the production of semiochemicals having pheromonal properties is discussed.

The skin, forming the boundary of a multicellular organism with its outside world, assists in communication with other organisms. To that end it may excrete substances that function as pheromones (Pfeiffer, 1982). This is, e.g., the case in *Ictalurus* species (Todd *et al.*, 1967; Richards, 1974). In other fish species, e.g., *Gobius jozo* (Colombo *et al.*, 1982), zebrafish, *Brachydanio rerio* (Van den Hurk and Lambert, 1983; Lambert *et al.*, 1986; Van den Hurk *et al.*, 1987), and African catfish *Clarias gariepinus* (Lambert *et al.*, 1986; Resink *et al.*, 1987), pheromones of gonadal origin can induce breeding behavior and spawning. In the goby and the zebrafish these pheromones are steroid glucuronides. In the African catfish steroid glucuronides are being produced by the male seminal vesicles and the female ovaries (Schoonen and Lambert, 1986b; Schoonen *et al.*, 1987a,c), and preliminary investigations by Resink and Van den Hurk point to these water-soluble steroids as sex pheromones in this species.

The gonads, however, may not be the only organ that produces and excretes pheromonal steroid glucuronides. Indeed, *in vitro* studies by Hay *et al.* (1976) and Soivio *et al.* (1982) indicate the presence of the enzymes 3α-hydroxysteroid dehydrogenase (3αHSD), 17βHSD, and 5α-reduc-

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tase in the skin of the rainbow trout *Salmo gairdneri* and the brown trout, *Salmo trutta lacustris*, respectively, and water-soluble steroid conjugates were found in skin incubations of brown trout. This points to the skin as a possible source of pheromonal steroid conjugates. In evaluating such an hypothesis for the African catfish, the first thing to do is study steroid metabolism in the skin. To that end, skin fragments of *C. gariepinus* are studied biochemically by incubations *in vitro* using six different radiolabeled steroid precursors, and enzymehistochemically by demonstrating enzyme activities involved in the synthesis of steroids and glucuronic acid.

MATERIAL AND METHODS

Animals. African catfish, *C. gariepinus*, were reared from eggs to maturity in the laboratory. The breeding techniques and hatchery conditions have been reported by De Leeuw *et al.* (1985). Pieces (5 × 7 cm) of skin taken from the lateral side of the fish from dorsal to ventral were obtained from adult male fish (weight 500 g), cleaned from adipose tissue, and prepared for *in vitro* incubations to study the bioconversions of steroids and for histological and enzymehistochemical studies.

Histology and enzymehistochemistry. Enzymehistochemical studies were carried out on 10-μm-thick transversal sections of tissues that were immediately frozen with CO_2 after killing the fish, and cut with a cryostat microtome (Minotome TH, Damon/IEC Division) at −25°. The enzymes 3β- and 3α-hydroxysteroid dehydrogenase were demonstrated according to the method of Van den Hurk (1973) with epiandrosterone and etiocholanolone, respectively, as substrates. The incubation temperature was 30° and the incubation time 3 hr. 11β-Hydroxysteroid dehydrogenase (11β-HSD) was demonstrated with the same method, using 11β-hydroxyandrostenedione as substrate. 17β-Hydroxysteroid dehydrogenase (17β-HSD) was demonstrated according to the method of Kellog and Glenner (1960) with testosterone as substrate in a medium (pH 9.2) containing 0.1 mg/ml polyvinylpyrrolidone (M_r 125,000) instead of crystalline bovine albumin. Demonstration of UDP–glucose dehydrogenase (UDPGD) was carried out according to the method of Van den Hurk *et al.* (1987). Sections were also incubated in solutions lacking substrate or co-factor to control the specificity of the reaction. In addition, uridine-5′-diphosphoxylose (1 m*M*) was used as an inhibitor of the UDPGD reaction.

Alternate frozen sections and 7-μm-thick paraffin sections of tissues fixed in Bouin–Hollande were stained with Mayer's hemalum–eosin (Burck, 1981) for more detailed morphological information.

Chemicals. [7-^{3}H]Pregnenolone (9.4 Ci/mmol), [7-^{3}H]17α-hydroxyprogesterone (10.7 Ci/mmol), [7-^{3}H]androstenedione (9.2 Ci/mmol), [7-^{3}H]testosterone (12.4 Ci/mmol), and [7-^{3}H]dehydroepiandrosterone (14.5 Ci/mmol) were obtained from Radio Chemical Centre, Amersham (U.K.). [4-^{14}C]11β-hydroxyandrostenedione (55.1 mCi/mmol) was prepared from [4-^{14}C]cortisol with sodium bismuthate (Bush, 1961). The purity of these chemicals was checked by thin-layer chromatography (TLC) before use. Leibovitz L-15 medium was from Serva and Hepes was from Boehringer. The substrates used in the enzymehistochemical studies were from Merck. All chemicals used in the experiments were of analytical grade.

Chromatography. Thin-layer chromatography was carried out on precoated plates with Silica F254 (Merck A.G.) in saturated tanks with the following systems: I, toluene–cyclohexane (1:1); II, benzene–ethyl acetate (3:1); III, chloroform–ethanol (95:5); IV, diisopropylether–chloroform–hexane (7:2:1); V, ethylacetate–hexane–acetic acid (75:20:5). Carrier and reference steroids were identified by uv absorption (3-keto-Δ^4- steroids) or by spraying with primuline according to Wright (1971).

The relative R_f values [R_s(T)] are expressed against testosterone. Microchemical reactions, recrystallizations, and measurement of radioactive samples were carried out according to Schoonen and Lambert (1986a). Radioactive areas on TLC plates were located and quantified by means of a Berthold thin-layer chromatogram linear scanner (LB 2842). Only the radioactive areas having more than 1% of the total activity were identified.

Incubation procedure. The incubations were carried out with 0.5 g of skin cut into small pieces of ± 1 mm^2 in 2 ml Leibovitz medium fortified with 15 m*M* Hepes (pH 7.4) containing 2.1 μCi of the radiolabeled precursor dissolved in 100 μl of propyleneglycol. No cofactors were added. As the water temperature in which the animals lived was 26°, the incubations were carried out at this temperature under continuous shaking in an air atmosphere for 3 hr. The enzyme reactions were terminated by addition of 10 ml ethanol. All the incubations were carried out in duplicate using two fish.

Extraction. Before extraction, 25 μg of each of the following carrier steroids were added based on the results of steroid metabolism in testes and seminal vesicles (Schoonen and Lambert, 1986a,b, Schoonen *et al.*, 1987a,b). To incubations with pregnenolone and 17α-hydroxyprogesterone were added pregnenolone, 17α-hydroxypregnenolone, progesterone, 17α-hydroxyprogesterone, androstenedione, testosterone, 11β-hydroxyandrostenedione, 11β-hydroxytestosterone, and 11-ketotestosterone. 5β-Pregnane-3α,17α-

diol-20-one was added only to incubations with 17α-hydroxyprogesterone. To all the other incubations were added androstenedione, testosterone, 11β-hydroxyandrostenedione, 11β-hydroxytestosterone, 11-ketotestosterone, 5β-androstan-3α-ol-17-one (etiocholanolone), 5β-androstan-3β-ol-17-one (epietiocholanolone), 5β-androstane-3α,17β-diol, 5α-androstane-3α,17β-diol, 5β-androstane-3β,17β-diol, and 5β-dihydrotestosterone. In addition, dehydroepiandrosterone was added only to the incubations with [^{3}H]dehydroepiandrosterone.

The products from the incubations were extracted by ethanol (3 × 10 ml). The ethanol–medium mixture was evaporated to dryness under nitrogen and the residue was redissolved in 2 ml of distilled water. Dichloromethane was then added (3 × 10 ml) to extract the free steroids from the water. The combined extracts of the organic fraction were evaporated and the residue was dissolved in a few drops of dichloromethane–methanol (9:1). This final solution in total was subjected to TLC in system I to remove the nonpolar compounds from steroids. In this system the steroids remain on the baseline; therefore it was possible, after drying, to use the same plate for the first separation of steroids in system II.

The remaining water fractions, containing 10% or more of the original activity, were treated with β-glucuronidase of *Escherichia coli* (Schoonen and Lambert, 1986b) and free steroids were extracted with dichloromethane (3 × 10 ml) and separated by TLC as in the case of the original organic fraction.

RESULTS

Histology and Enzymehistochemistry

The skin of *C. gariepinus* has no scales and is composed of two distinct layers: the epidermis and, beneath this, the dermis (Fig. 1A). The epidermis is a stratified epithelium with numerous mucous glands and so-called club cells with two central nuclei. The dermis is a composite layer a.o. including connective tissue, muscle fibers, and capillary blood vessels (Fig. 1A). The deeper main portion of the dermis, the stratum compactum, consists of dense connective tissue with collagenous fibers running more or less parallel to the surface. Occasionally, bundles are oriented perpendicular. The outer surface of the dermis, the stratum vasculare, is in contact with the epithelium and consists of looser connective tissue with much thinner collagenous bundles. A network of fibroblasts is distributed between the collagenous bundles. They are more abundant in the outer layer than in the deeper layer of the dermis. Beneath the dermis is found the hypodermis, a narrow layer of loose connective tissue and subcutaneous adipose tissue.

3β-HSD could not be demonstrated in the skin, but weak 3α-, 11β-, and 17β-HSD activity was found in the epithelial cells (Fig. 1B). In these cells UDPGD activity was moderate (Fig. 1C). In addition, a moderate to strong UDPGD reaction was found in the fibroblasts of the dermis. Sections of skin that were incubated in substrate-free or co-factor-free media remained unstained. UDP–xylose completely inhibited the UDPGD reaction.

Incubation with [^{3}H]Pregnenolone

After chromatography in system II and scanning, only one radioactive area was observed in this incubation. After TLC in system III, acetylation, and recrystallization, it was confirmed that the single area was the precursor itself (Table 1), indicating that pregnenolone was not metabolized to any other steroid.

Incubation with [^{3}H]Dehydroepiandrosterone

The distributions of radioactivity between the organic and the water fractions were 78 an 22%, respectively. After separation in system II the organic fraction gave two areas. These two areas, after TLC in system III, corresponded to the carriers 5-androstene-3β,17β-diol and dehydroepiandrosterone. After acetylation and recrystallization these radioactive compounds were proven to be 5-androstene-3β,17β-diol and the precursor dehydroepiandrosterone (Table 1).

In the water fraction after glucuronidase treatment and extraction, only one radioactive area was found after TLC and scan-

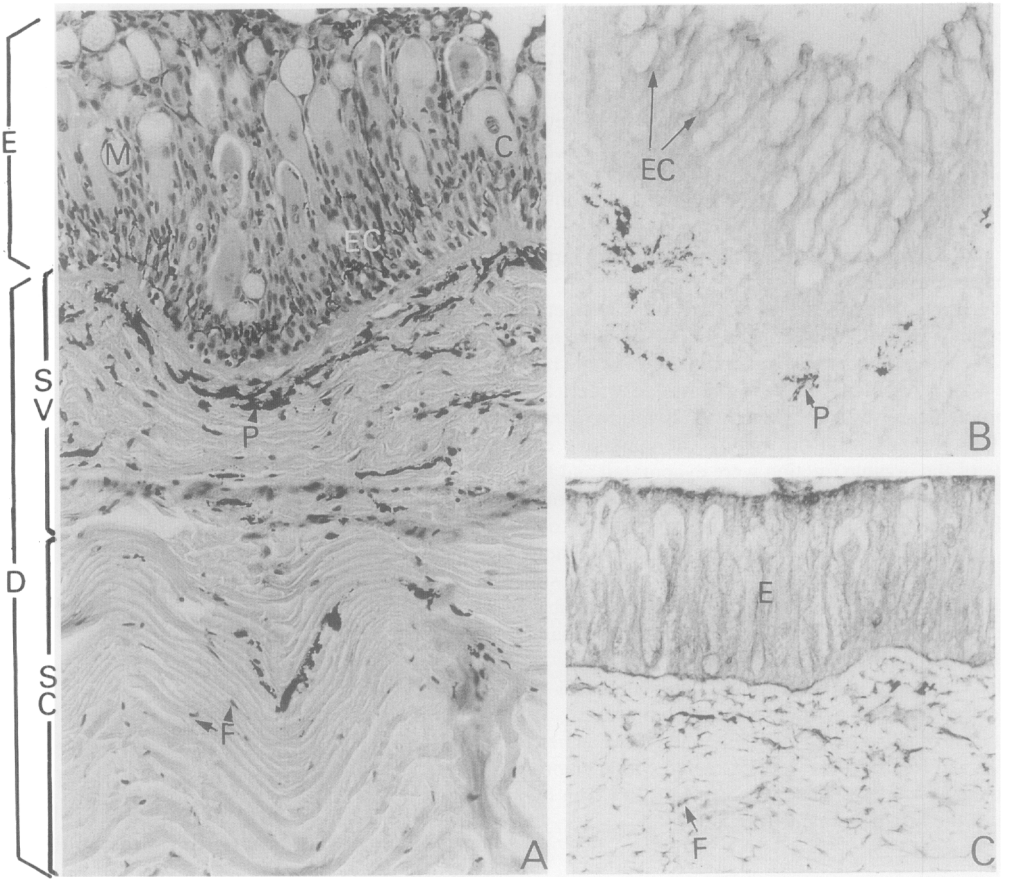

FIG. 1. Histological sections of the skin of *Clarias gariepinus*. (A) General structure. Hemalum–eosin, × 230. (B) 17β-Hydroxysteroid dehydrogenase, with a weak enzyme activity in epithelial cells. ×145. (C) UDP–glucose dehydrogenase. Enzyme activity is demonstrated in the epidermis and fibroblasts. ×145. C, club cells; D, dermis; E, epidermis; EC, epithelial cells; F, fibroblasts; M, mucous gland; P, pigment; SC, stratum compactum; SV, stratum vasculare.

ning. The substance was confirmed as 5-androstene-3β,17β-diol after TLC in system III, derivatization, and recrystallization (Table 1).

Incubation with [^3H]17α-Hydroxyprogesterone

Of the total radioactivity, 90% was found in the organic fraction. After separation by TLC in system II, four radioactive areas were found in this fraction (Fig. 2) with the following R_s(T) values: area A, 0.09 to 0.22; area B, 0.43 to 0.56; area C, 1.10 to 1.22; and area D, 1.31 to 1.44. In area A, after rechromatography in systems III and IV, two radioactive areas were observed, one corresponding to 5β-pregnane-3α,-17α,20α-triol and the other to 5β-pregnane-3α,17α,20β-triol. The areas B, C, and D were eluted and rechromatographed separately in system III and, after derivatization and recrystallization, were found to be 5β-pregnane-3α,17α-diol-20-one, 17α-hydroxyprogesterone, and 5β-pregnan-17α-ol-3,20-dione, respectively (Table 1).

The water fraction after treatment with β-glucuronidase, extraction, and chromatography in system II showed two areas with comparable R_s(T) values as the areas A and B of the original organic fraction. These compounds were found to be 5β-

TABLE 1

IDENTIFICATION OF INCUBATION PRODUCTS OF SKIN OF THE AFRICAN CATFISH, *Clarias gariepinus*, *IN VITRO*

Substrate	Metabolite	Percentage of total activity	TLC systems[a]			Crystallizations (dpm/mg)			
						Original	1	2	3
Pregnenolone	Pregnenolone	100	II (4×)	III	A^+,II (2×)	290,055	271,656	255,443	252,700
Dehydroepiandrosterone	Dehydroepiandrosterone	45	II (4×)	III	A^+,II (2×)	58,351	54,206	53,378	51,360
	5-Androstene-3β,17β-diol	18	II (4×)	III	A^+,II (2×)	1,224	1,094	1,129	1,097
	5-Androstene-3β,17β-diol-GL	22	II (4×)	III	A^+,II (2×)	6,818	4,526	4,400	4,133
17α-Hydroxyprogesterone	5β-Pregnane-3α,17α,20α-triol	2	II (4×)	V (2×)	A^+,II (2×)	—	—	—	—
	5β-Pregnane-3α,17α,20β-triol	6	II (4×)	V (2×)	A^+,II (2×)	43,935	43,791	42,154	40,196
	5β-Pregnane-3α,17α-diol-20-one	48	II (4×)	III	A^+,II (2×)	152,739	152,241	152,030	146,881
	17α-Hydroxyprogesterone	15	II (4×)	III	A^-,II (2×)	20,401	19,146	18,332	19,233
	5β-Pregnan-17α-ol-3,20-dione	7	II (4×)	III	A^-,II (2×)	7,477	7,173	7,221	7,313
	5β-Pregnane-3α,17α,20β-triol-GL	5	II (4×)	V (2×)	A^+,II (2×)	5,882	5,196	5,396	5,287
	5β-Pregnane-3α,17α-diol-20-one-GL	5	II (4×)	III	A^+,II (2×)	5,150	4,726	4,979	4,996
Androstenedione	Testosterone	4	II (4×)	III	A^+,II (2×)	26,160	20,327	19,892	20,244
	Etiocholanolone	8	II (4×)	III	A^+,II (2×)	18,140	16,936	17,531	17,040
	Androsterone	3	II (4×)	III	—	4,945	4,424	4,138	4,236
	Androstenedione	17	II (4×)	III	A^-,II (2×)	43,374	39,718	42,913	42,054
	5α-Androstane-3α,17β-diol	2	II (4×)	III	A^+,II (2×)	3,565	1,083	1,164	1,129
	5α-Androstane-3,17-dione	3	II (4×)	IV (3×)	A^-,II (2×)	1,685	1,571	1,627	1,665
	5β-Androstane-3,17-dione	2	II (4×)	IV (3×)	A^-,II (2×)	—	—	—	—
	Testosterone-GL	8	II (4×)	III	A^+,II (2×)	7,512	6,883	6,605	6,278
	5β-Dihydrotestosterone-GL	46	II (4×)	III	A^+,II (2×)	28,268	27,152	26,432	26,167
11β-Hydroxyandrostenedione	11β-Hydroxytestosterone	8	II (4×)	III	O^+,II (2×)	—	—	—	—
	11-Ketotestosterone	10	II (4×)	III	O^+,II (2×)	—	—	—	—
	11β-Hydroxyandrostenedione	67	II (4×)	III	A^-,II (2×)	905	857	852	871
	11-Ketoandrostenedione	6	II (4×)	III	A^-,II (2×)	151	149	144	150
Testosterone	Testosterone	13	II (4×)	III	A^+,II (2×)	24,614	22,589	23,396	22,595
	Etiocholanolone	3	II (4×)	III	A^+,II (2×)	6,586	5,912	5,361	5,577
	Androstenedione	2	II (4×)	III	A^-,II (2×)	15,805	14,216	14,866	15,502
	Testosterone-GL	23	II (4×)	III	A^+,II (2×)	22,314	22,228	21,943	21,584
	5β-Dihydrotestosterone-GL	45	II (4×)	III	A^+,II (2×)	50,424	48,022	48,276	47,030
	5β-Androstane-3β,17β-diol-GL	2	II (4×)	III	A^+,II (2×)	11,014	10,279	9,792	9,666
	5β-Androstane-3α,17β-diol-GL	4	II (4×)	III	A^+,II (2×)	5,992	4,327	4,462	4,409

[a] For explanation of Roman numerals, see Materials and Methods. A^+, positive acetylation. A^-, negative acetylation. GL, glucuronide.

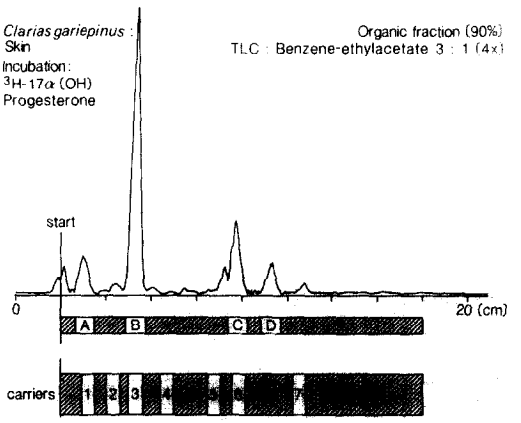

FIG. 2. Radiochromatogram of the organic fraction of skin incubation of *Clarias gariepinus* with [^{3}H]17α-hydroxyprogesterone. Carriers: (1) 11β-hydroxytestosterone, (2) 11-ketotestosterone, (3) 5β-pregnane-3α,17α-diol-20-one, (4) 11β-hydroxyandrostenedione, (5) testosterone, (6) 17α-hydroxyprogesterone, (7) androstenedione. Areas A–D were eluted.

pregnane-3α,17α,20α-triol and 5β-pregnane-3α,17α-diol-20-one (Table 1).

Incubation with [^{3}H]Androstenedione

The incubation with [^{3}H]androstenedione resulted in 46% of the radioactivity in the organic fraction and 54% in the water fraction. The organic fraction gave six radioactive areas after separation by TLC in system II with the following R_s(T) values: areas A, 0.93 to 1.06; area B, 1.06 to 1.21; area C, 1.28 to 1.38; area D, 1.38 to 1.49; area E, 1.49 to 1.60; and area F, 1.78 to 1.96 (Fig. 3A). After rechromatography in system III the following steroids could be identified: area A, testosterone and 5α-androstane-3α,17β-diol; area B, etiocholanolone; area D, androsterone; and area E, androstenedione. The compound of area C could not be identified, while rechromatography of area F in systems III and IV resulted in two substances, 5α-androstane-3,17-dione and 5β-androstane-3,17-dione (Table 1).

After treatment with β-glucuronidase and subsequent extraction with dichloromethane, 95% of the radioactivity of the water fraction passed to the organic phase,

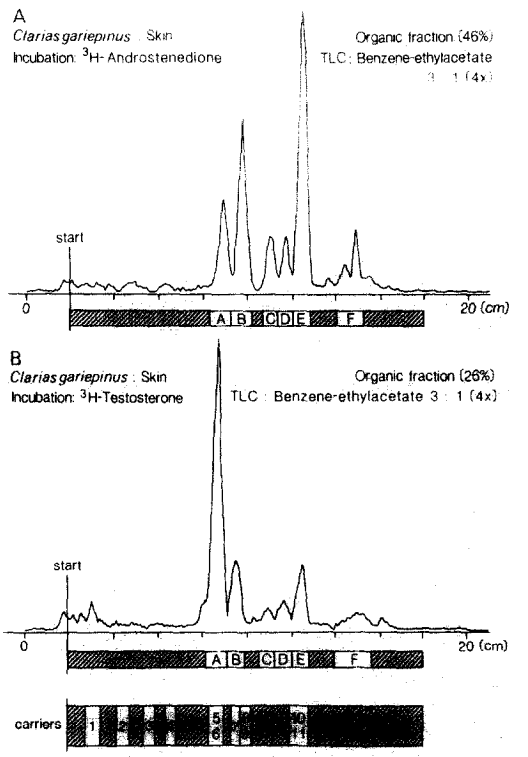

FIG. 3. Radiochromatograms of the organic fractions of skin incubations of *Clarias gariepinus* with [^{3}H]androstenedione (A) and [^{3}H]testosterone (B). Carriers: (1) 11β-hydroxytestosterone, (2) 11-ketotestosterone, (3) 5β-androstane-3α,17β-diol, (4) 11β-hydroxyandrostenedione, (5) 5α-androstane-3α,17β-diol, (6) testosterone, (7) etiocholanolone, (8) 5β-androstane-3β,17β-diol, (9) 5β-dihydrotestosterone, (10) epietiocholanolone, (11) androstenedione. Areas A–F were eluted.

indicating that the steroid conjugates present were glucuronides. The free steroids, when subjected to TLC in system II, resulted in two radioactive areas. The first area R_s(T), 0.9 to 1.1, after rechromatography in system III gave two compounds; one was 5α-androstane-3α,17β-diol and the other testosterone. The second area R_s(T), 1.1 to 1.3, was found to be 5β-dihydrotestosterone (Table 1).

Incubation with [^{14}C]11β-Hydroxyandrostenedione

In this incubation 95% of the radioactivity was recovered in the organic frac-

tion. Following separation by TLC in system II, four radioactive areas were obtained. The compounds of these zones were rechromatographed in system III and were identified as 11β-hydroxytestosterone, 11-ketotestosterone, 11β-hydroxyandrostenedione, and 11-ketoandrostenedione. The identity of the first two compounds was confirmed by oxidation and rechromatography of the product (11-ketoandrostenedione). The identity of the latter two compounds was confirmed by acetylation and recrystallization (Table 1).

Incubation with [^3H]Testosterone

In the testosterone incubation, 26% of the total radioactivity was found in the dichloromethane phase and 74% was found in the water fraction. After separation by TLC in system II, the organic fraction gave five different radioactive areas (Fig. 3B). Area A with R_s(T) 0.91 to 1.06 corresponded to the precursor testosterone, and area B with R_s(T) 1.06 to 1.18 was found to be etiocholanolone. Areas C and D with R_s(T) 1.26 to 1.37 and 1.37 to 1.49, respectively, were not identified. After TLC in system III, area E with R_s(T) 1.49 to 1.60 corresponded to androstenedione (Table 1). Area F with R_s(T) 1.76 to 2.01 also was not identified.

From the fraction treated with β-glucuronidase, 95% of the radioactivity was recovered as free steroids. After separation by TLC in system II, three different radioactive areas were obtained. The first area was identified as 5β-androstane-3α,17β-diol. The second area, after rechromatography in system III, gave two peaks, one corresponding to the steroid testosterone and the other to 5β-androstane-3β,17β-diol. The third area was found to be 5β-dihydrotestosterone. All the compounds were confirmed by acetylation and crystallization (Table 1).

DISCUSSION

The general structure of the skin of *C. gariepinus* corresponds to that of other teleost fish (Kann, 1927; Pfeiffer, 1960, 1963, 1967; Bremer, 1972; Yamazaki, 1972). The club cells are the most characteristic elements in the epithelium of fish belonging to the Ostariophysi. These club cells are known as alarm substance cells (Pfeiffer, 1967) that do not open onto the surface, but release their contents only when the skin is injured.

The results of the biochemical study have shown that in the skin of the catfish, Δ^4 steroids are metabolized more actively than are Δ^5 steroids. Moreover, it appears that in skin incubations, a variety of androgens can be found as water-soluble steroid glucuronides. The results of the incubations with different precursors indicate that the skin cannot be considered as a steroidogenic organ, but that metabolism in the skin of circulating steroids cannot be excluded. A key enzyme in steroid biosynthesis is 3β-hydroxysteroid dehydrogenase. This enzyme could be demonstrated neither enzymehistochemically nor biochemically. Pregnenolone and dehydroepiandrosterone were not converted to progesterone and androstenedione, respectively. The enzymes of the Δ^4 and Δ^5 route, such as 17α-hydroxylase and C_{17-20}-lyase, are also absent, for 17α-hydroxyprogesterone was not converted to androstenedione. The conversion products of 17α-hydroxyprogesterone, androstenedione, 11β-hydroxyandrostenedione, and testosterone indicate that the main metabolic enzymes present in the skin were 5α-reductase, 5β-reductase, 3α-HSD, 11β-HSD, 17β-HSD, 20β-HSD, and steroid–glucuronosyl transferase. From enzymehistochemical studies it appeared that these metabolic enzymes are localized in the epithelial cells of the skin. 11β-Hydroxyandrostenedione was not reduced, although the enzymes 5α- and 5β-reductase were present. This indicates a substrate specificity of these enzymes. A functional group at the C_{11} position might prevent reduction. Seminal vesicles of the

catfish incubated with 11β-hydroxyandrostenedione create the same impression. Although the reductase enzymes are highly active, from what can be concluded from androstenedione incubations, only a minor reduction of 11β-hydroxyandrostenedione could be observed (Schoonen *et al.*, 1987a).

The presence of some of the above-mentioned metabolic enzymes, i.e., 3α-HSD, 17β-HSD, and 5α-reductase, was also demonstrated in the skin of *S. gairdneri* (Hay *et al.*, 1976) and *S. trutta lacustris* (Soivio *et al.*, 1982).

These studies did not, however, indicate the presence of 5β-reductase, 11β-HSD, and 20β-HSD, which were found to be active in the skin of African catfish. In catfish there also was a high conversion of androgens into water-soluble steroid conjugates, in particular the glucuronides of testosterone and 5β-dihydrotestosterone. The epithelial cells can be considered as the source of these steroid glucuronides, since the presence of UDPGD in these cells in combination with enzymes involved in steroid metabolism (Van den Hurk *et al.*, 1987b). Considerable quantities of water-soluble compounds were also found in the incubations of skin of the brown trout (Soivio *et al.*, 1982) but these compounds were not identified. By contrast, in the skin of the rainbow trout, Hay *et al.* (1976) found only traces of water-soluble compounds.

The bioconversion of steroids by epithelial cells in the skin may be considered as a physiological process, since the appropriate precursors such as 17α-hydroxyprogesterone, androstenedione, and testosterone are produced in the African catfish in testes and seminal vesicles (Schoonen and Lambert, 1986a, b; Schoonen *et al.*, 1987a, b). The presence of these steroids was also demonstrated in the blood (De Leeuw *et al.*, 1986).

Hay *et al.* (1976) have suggested that the glucuronides found in the rainbow trout skin could be detoxification products. This may also be true in the case of *C. gariepinus*. However, the possibility of steroid glucuronides, formed in the skin, playing a significant role as sex pheromones cannot be ruled out. In the African catfish, steroid glucuronide fractions, obtained from seminal vesicles and ovaries, possess pheromonal functions (Lambert *et al.*, 1986; Resink *et al.*, 1987). Pheromonal properties of steroid glucuronides were also demonstrated in zebrafish, *B. rerio* (Van den Hurk and Lambert, 1983) and in *G. jozo* (Colombo *et al.*, 1980).

Production of pheromones by the skin of fish has already been suggested by Todd *et al.* (1967). They studied the behavior of yellow bullhead, *Ictalurus natalis*, and demonstrated that these animals know conspecifics by their pheromones. The main source of the intraspecific chemical stimuli involved in recognition was suggested to be the mucus. Using extracts from the skin, the urophysis, and the urinary bladder, Richards (1974) could demonstrate that the channel catfish, *Ictalurus nebulosus*, can discriminate between two conspecifics. Although the chemical identity of the extracts is not known, these observations clearly suggest that the skin of catfish species is involved in the production of substances with pheromonal properties.

In the African catfish steroid glucuronides can act as pheromones (Lambert *et al.*, 1986; Resink *et al.*, 1987). The present results indicate that the skin, and in particular its epithelial cells, is capable of synthesizing steroid glucuronides. Thus, epithelial cells of the skin might be involved in the production of semiochemicals with pheromonal properties.

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