Roux' Archiv für Entwicklungsmechanik, Bd. 144, S. 257-264 (1950).

(From the Department of Zoology, University of Calcutta, Calcutta, India.)

RESPONSES OF THE GENITAL SYSTEM AND THE URINOGENITAL PAPILLA OF THE FEMALE CATFISH, *HETEROPNEUSTES FOSSILIS* (BLOCH) TO SEX HORMONES.

By

AMIYA B. KAR, Ph. D., and ASOK GHOSH, M. Sc.

With 10 text figures.

(Eingegangen am 5. Juli 1949.)

Introduction.

A great amount of work has been done on the influence of sexual hormones on the primary and secondary sexual characters of mammals, birds and amphibians. Unfortunately, comparable reports on such investigations in fishes are meager. That the sex hormones have activity in the latter group, in one manner or another, has been demonstrated in *Lebistes reticulatus* (BERKOWITZ 1937, 1938 and 1941; EVERSOLE, 1939 and 1941; SCOTT 1941 and 1944), *Gambusia affinis* (TURNER 1941 and 1942), *Xiphophorus helleri* (RÉGNIER 1937; BALDWIN and GOLDIN 1940; WITSCHI and CROWN 1937; NOBLE and BOURNE 1940), *Tilpia macrocephala* (ARONSON and HOLTZTUCKER 1947), *Rhodeus amarus* (WUNDER 1931), *Chrosomus erythrogaster* (SAPHIR 1934), *Platypoecilus maculatus* (COHEN 1946) and *Salmo irideus* (PADOA 1937). Much of this and other work have been summarized and discussed broadly by WITSCHI (1939, 1942). Curiously, in most of these the studies the attention has mostly been focussed on the responses elicited by the sex hormones in the secondary sexual characters, while the effects of these substances on the gonads themselves, have largely been ignored.

In view of the above, the present experiment was planned to study the action of sex hormones on the female genital system of a catfish, *Heteropneustes fossilis* (BLOCH). A focal point of the present study has been the histological changes elicited in the gonads, gonoducts, and the urinogenital papilla of this species after hormonal treatments.

Procedure.

The female catfishes, *Heteropneustes fossilis* (BLOCH) involved in the present experiment were purchased from a local market. These were brought to the Fishery Laboratory, University College of Science, where they were kept in glass aquaria and maintained under uniform conditions throughout the duration of the experimental period. The initial body weight and length of each fish were taken at the commencement of the experiment, and the final weight was noted on the day of autopsy.

The testoid, testosterone propionate in sesame oil was administered intraperitoneally. Four injections of this hormone were made over a period of 16 days

(April 8 to April 23, 1949) and the total amount of androgen injected was 10 mg. An equal amount of diethylstilbestrol was injected in the similar manner and over the same period of time into a second group of fishes. Uninjected specimens served as the controls.

All the fishes were autopsied on the day following the last injections. The ovaries were carefully dissected out and weighed to the nearest mgm. Pieces of the oviduct, ovary and the urinogenital papilla were fixed in alcoholic Bouin's fluid for histological studies. The sections were prepared by the paraffin method and stained with Delafield's haematoxylin followed by eosin.

Experimental Results.

A. Macroscopic observations.

Gross examinations at autopsy reveals pronounced and consistent inhibition of the ovaries of the experimental females. However, the degree of inhibition is more marked in the estrogen recipients than in the androgen treated ones. The quantitative data on the ovary, presented in table 1 will also indicate that the absolute and relative weights of this organ in the hormone treated fishes are significantly lower as compared with the ovarian weights of the control specimens.

Table 1. *Ovarian weights in control and hormons treated Heteropneustes fossilis.*

Treatment	Number of fishes	Ovarian weight		Body weight at autopsy	Length at autopsy
		Absolute	Relative ÷		
		Gm.	per cent.	Gm.	Gm.
Uninjected controls	9	2,5 ± 0,87*	3,12	78,3 ± 6,72	24,7 ± 0,77
Estrogen treated .	6	0,2 ± 0,04	0,2	68,0 ± 8,5	24,0 ± 1,2
Androgen treated .	6	0,4 ± 0,53	0,5	72,0 ± 9,5	23,8 ± 1,1

* Standard deviation. ÷ Organ weight expressed as percentage of body weight.

The ovaries of the control females are elongated massive structures which practically fill the entire extent of the peritoneal cavity. These exhibit a markedly hyperemic condition and are full of ripe ova (Fig. 1a). The gross appearence is typical of a fish ovary in breeding condition. The two ovaries are fused posteriorly and the single oviduct is an extremely short tube.

In this species of catfish the females are distinguished from the males by the morphology of the urinogenital papilla. The latter is a muscular sac-like structure located on the mid-ventral surface of the body and a short distance caudal to the anal opening. In the males the papilla is pointed in shape and has an opening at the tip, while in the females it is much broader and possesses a median cleft (MOOKERJEE et al, 1941). The urinogenital papilla of the control females is highly vascularized and presents a swollen appearence (Fig. 2a). These conditions of the papilla are encountered in the females during the spawning season (MOOKERJEE *et al*, 1941).

In contrast to the above conditions, the ovaries of the treated fishes present an immature condition. These organs are much smaller in size and are markedly non-vascular (Fig. 1b and c). The oviduct of the

experimental fishes also appear much less conspicuous in comparison with that of the control fishes. However, it should be noted that macros-

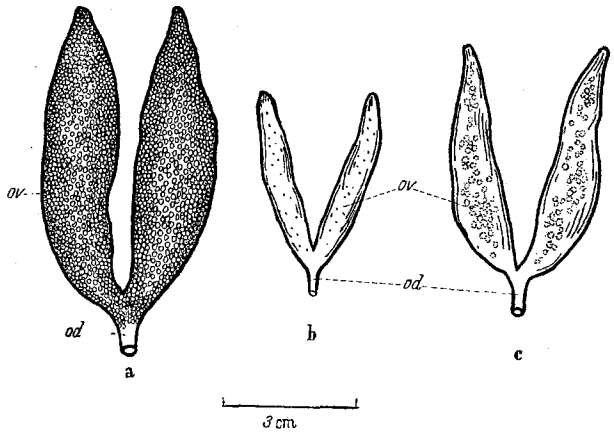

Fig. 1. Reproductive organs of female Heteropneustes fossilis (ventral views). a—control; b—estrogen treated; c—androgen treated. Ov—Ovar ; Od—oviduct.

copic pictures gave a clear indication that the inhibitory consequences of the hormonal treatments are more pronounced in the astrogen recipi-

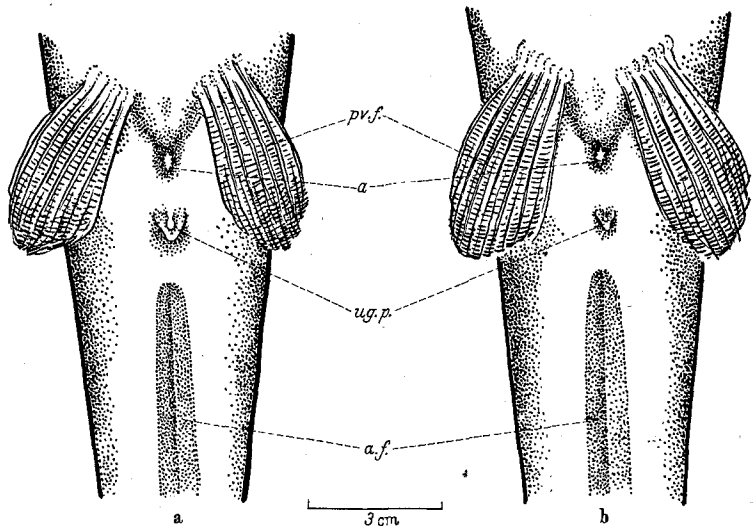

Fig. 2. Ventral view of Heteropneustes fossilis showing the urinogenital papilla. a—control; b—estrogen treated specimen. Ug. p. Urinogenital papilla; a, anus; a.f, anal fin; pv. f, pelvic fin.

ents than in the androgen treated ones (Compare Fig. 1b with 1c).

Concomittant with the changes in the reproductive organs the urinogenital papilla also shows noteworthy changes after hormonal treatments. The papilla becomes smaller and strikingly non-vascular appearence (Fig. 2b) in contrast to the conditions in the control catfishes.

The gross morphology of the papilla in the estrogen and androgen treated series are however, more or less similar.

B. Microscopic observations.

Control females. Examination of the serially sectioned ovaries shows that the organ is full of mature ova. The yolk is present in large masses,

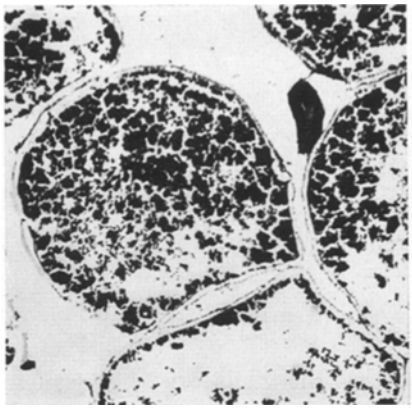

Fig. 3. Photomicrograph of the section through the ovary of a control catfish (× 45).

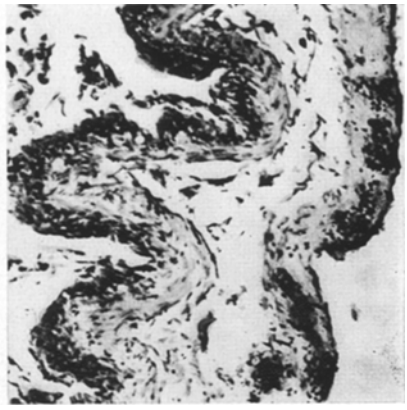

Fig. 4.—Photomicrograph of the section through the oviduct of a control catfish (× 170). Note the villus like folds of the mucosa.

Fig. 5. Photomicrograph of the section through the urinogenital papilla of a control catfish (× 170). Note the pigment layer, and thick blood vessels in the subcutaneous region.

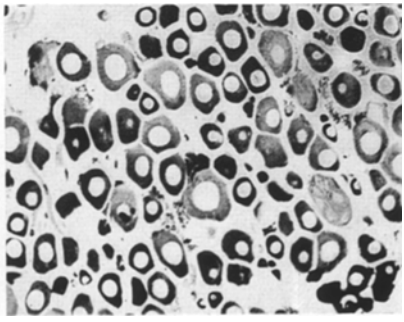

Fig. 6. Photomicrograph of the section through the ovary of an estrogen treated fish (× 45). Compare with figs. 3 and 9.

and the follicular epithelium appears to be of a secretory nature (Fig. 1). The interstitial tissue is practically absent and the interfollicular spaces are empty. The largest follicle averages $1{,}0 \pm 0{,}06$ mm in diameter.

The oviduct reveals a prominent lumen lined by villus-like folds of the mucosa. The mucosa is made up of glandular cells, more or less spherical in shape which exhibit a crowded appearence on the mucosal folds (Fig. 4). The submucosa extends throughout the axial portion of the folds. The space between the muscularis and the submucosa is filled with collagenous tissue with condensations of fibro-

blasts at certain locations. The blood vessels are prominent in the muscularis beneath the stroma (Fig. 4).

The urinogenital papilla discloses that it has a cutaneous covering which is seperated from the subcutaneous region by a thick layer of pigment (Fig. 5). The subcutaneous region is made up mostly of muscles with very little connective tissue interposed. This region is very richly vascularized (Fig. 5).

Estrogen treated females. The ovary shows an immature condition. The entire organ is full of non-yolked, immature oocytes (Fig. 6). Any given oocyte resembles a typical cell with irregular outline and a slightly

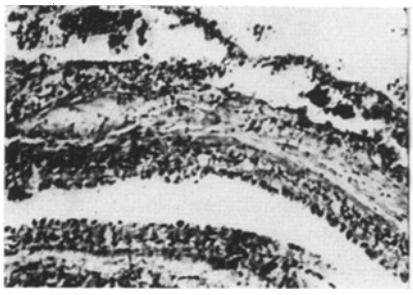

Fig. 7. Photomicrograph of the section through the oviduct of an estrogen treated fish (× 170). Compare with figs. 4 and 10.

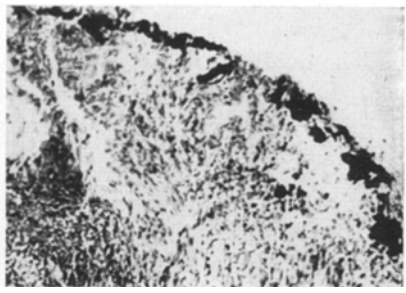

Fig. 8. Photomicrograph of the section through the urinogenital papilla of an estrogen treated female (× 170). Compare with fig. 5.

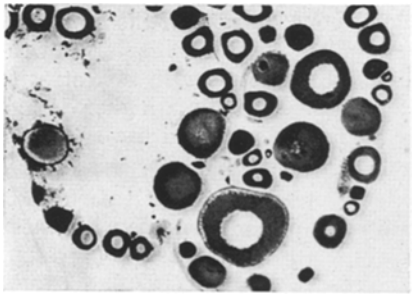

Fig. 9. Photomicrograph of the section through the ovary of an androgen treated fish (× 45).

Fig. 10. Photomicrograph of the section through the oviduct of an androgen treated specimen (× 170). Note the slight elevations of the mucosa.

excentrically placed nucleus. A prominent karyosome is present in the nucleus of some oocytes. There is not even the slightest indication of yolk deposition in any of the oocytes, and this pronounced lack of cytosomal differentiation is definitely reflected in the size of these cells, the largest of which averages 0,18 ± 0,02 mm as against 1,0 ± 0,06 mm. for the controls.

The oviduct exhibits a small lumen lined by the mucosa. The latter is lined by cells of non-secretory nature. The villus-like folds, charateristic of the mucosa of the control fishes are totally absent (Fig. 7). The submucosa presents the usual appearence of a connective tissue

layer. The muscularis can be identified as an extremely thin peripheral layer and the collagenous tissue is absent between the muscularis and the submucosa.

The urinogenital papilla shows noteworthy histological alterations. The cutaneous layer appears extremely inconspicuous and the pigment layer shows a diffused condition (Fig. 8). The subcutaneous region is remarkebly non-vascular (Compare fig. 8 with fig. 5).

Androgen treated females. The histological features of the ovary losely approach those of the estrogen treated specimens. However, some of the oocytes appear to show signs of cytosomal differentiation (Fig. 9). This is expressed in the size of the largest oocyte which averages $0{,}26 \pm 0{,}03$ mm in diameter.

The oviduct *reveals* a small lumen lined by the mucosa. The mucosal folds appear as slight elevations of the submucosa (Fig. 10) and the cellular features of the former are comparable with those of the estrogen treated females. The muscularis, submucosa, and the collagenous tissue closely approach those of the oviduct of the control fishes.

The histological observations confirmed the macroscopic findings, namely, that the features of urinogenital papilla in the androgen and estrogen treated females are more or less similar.

Discussion.

From the data presented in this report, there seems to be no doubt that treatments with gonadal hormones significantly provoked ovarian inhibition in the catfish. The histological consequences of the hormonal administration were expressed in the suppression of the follicular growth. However, the degree of inhibition was more pronounced in the estrogen recipients than in the androgen treated ones. Possibly, a higher dosage level of the androgen or a longer treatment period would have induced further inhibitory changes in the ovary.

With regard to the inhibitory effects of the testoid treatment on the ovary the results obtained here, are not out of keeping with those reported for *Salmo irideus* (PADOA 1937), *Xiphophorus helleri* (WITSCHI and CROWN 1937) and *Lebistes reticulatus* (EVERSOLE 1939). BERKOWITZ (1938) observed that the estrogenic hormone had no effect on the ovary of the latter species. However, in the present material a marked ovarian hypoplasia was noted after estrogen administration. This difference in the ovarian response to estrogen in the two fishes may be ascribed to a large extent to their specific difference. That the ovaries of various species of birds (*vide* KAR 1947*a* and *b*, 1948, 1949*a* and *b*) and mammals (*vide* BURROWS 1945) react in different manner to sex hormones has been adequately demonstrated. It is not unlikely that further studies with different species of catfish may show the same variability in ovarian response to sex hormones. However, the ovarian inhibition, as reported in this study, leads to the conclusion that the release of gonadotrophic hormones by the anterior hypophysis was suppressed as a result of hormonal treatments.

The inhibitory changes elicited in the ovaries by the gonadal hormones were closely correlated with likewise changes in the oviduct. These changes were reflected in the marked histological alternations in the latter organ. Thus, the conversion of the mucosa from villus-like folds to practically a flat layer was an item of considerable interest. The non-secretory nature of the mucosal epithelium in the experimental fishes was an unmistakable histological change. To these may be added another interesting feature, and that is the hypoplasia of the submucosa.

Now, a question may be raised as to whether the action of sex hormones on the oviduct was independent of the presence of the ovary. Judging from the data on the reactions of the catfishes to hormonal treatments, it appears extremely doubtful that the estrogen and the testoid acted directly on the oviduct. This can be gauged from the fact that in every instance of oviducal hypotrophy the ovary was also inhibited. It seems not unlikely, therefore, that the responses of the oviduct in the treated fishes were induced by the hormonally acted ovary through the inhibition of the hypophyseal gonadotrophic activity.

A further point of considerable interest was the histological changes in the urinogenital papilla of the hormone treated specimens. Here also, the changes were indicative of an inhibitory action of the steroids and appeared to be closely correlated with changes of similar nature in the ovary. The changes in the urinogenital papilla as reported in this study, prove beyond doubt that this structure in the female catfish is a true secondary sexual character and is under the estrogenic control of the ovary.

Summary.

Intraperitoneal injections of diethylstilbestrol and testosterone propionate into the catfish, *Heteropneustes fossilis* (BLOCH) elicited pronounced changes in the female reproductive system and the urinogenital papilla. Considerable gonadal and oviducal hypotrophy occurred in the hormone recipients. Marked histological alterations were also encountered in the genital system of the experimental females. Another interesting response was the inhibition of the urinogenital papilla of the treated fishes. It is suggested that the changes in the ovary and the oviduct were induced by the sex hormones throught the inhibition of the gonadotrophic activity of the anterior hypophysis. The changes in the urinogenital papilla proved that this structure in the female catfish is a true secondary sexual character and is under the estrogenic control of the ovary.

Zusammenfassung.

Intraperitoneale Injektionen von Diäthylstilböstrol und von Testosteronpropionat haben bei Heteropneustes fossilis ausgesprochene Veränderungen an den weiblichen Geschlechtsorganen und an der Urogenitalpapille zur Folge. Bei den Hormonempfängern kommt es zu einer beträchtlichen Hypotrophie der Keimdrüse wie des Ovidukts. Im Genitalsystem der Versuchstiere sind auch deutliche histologische Veränderungen nachzuweisen. Eine bemerkenswerte Folgeerscheinung

ist die Hemmung der Urogenitalpapille der behandelten Fische. Es ist anzunehmen, daß die Veränderungen im Eierstock und im Eileiter durch die Geschlechtshormone über eine Hemmung der gonadotropen Aktivität der Hypophyse veranlaßt werden. Die Veränderungen an der Urogenitalpapille beweisen, daß dieses Organ beim weiblichen Heteropneustes den Charakter eines wahren sekundären Geschlechtsmerkmales besitzt und unter der östrogenen Kontrolle des Eierstocks steht.

Acknowledgments.

The authors wish to express their indebtedness to Professor H. K. MOCKERJEE for providing facilities for research. Grateful acknowledgment is made to Dr. E. MOLDANODO of the Schering Corporation, Bloomfield, New Jersey, USA., for the generous contribution of testosterone propionate used in this study. Sincere thanks are due to Mr. P. C. PATHAK of the Pharmacognosy Laboratory, Govt. of India, for the photomicrographs which illustrate this paper.

Literature.

ARONSON, D. a. M. HOLTZTUCKER: Anat. Rec. **99**, 572 (1947). — BALDWIN, F. M. a. H. S. GOLDIN: Proc. Soc. exper. Biol. a. Med. **42**, 813 (1940). — BERKOWITZ, P.: Proc. Soc. exper. Biol. a. Med. **36**, 416 (1937). — Anat. Rec. **71**, 161 (1938). — J. exper. Zool. **87**, 233 (1941). — BURROWS, H.: Biological Actions of sex hormones. Cambridge Univ.-Press 1945. — COHEN, H.: Zoologica **31**, 121 (1946). — EVERSOLE, W. J.: Endocrinology **25**, 328 (1939); **28**, 603 (1941). — KAR, A. B.: Poultry Sci. **26**, 352 (1947*a*). — Anat. Rec. **97**, 175 (1947*b*). — Proc. Zool. Soc. Bengal. **1**, 81 (1948); **2**, 1 (1949*a*). — Ind. Vet. J. **25** (1949b. In press). — MOOKERJEE, H. K., S. R. MAJUMDER a. B. DAS GUPTA: Ind. J. Vet. Sci. a. Anim. Husb. **11**, 334 (1941). — NOBLE, G. K. a. R. BOURNE: Anat. Rec. **78**, 147 (1940). — PADOA, E.: Monit. Zool. Ital. **48**, 195 (1937). — RÉGNIER, M.: C. r. Acad. Sci. **205**, 451 (1937). — SAPHIR, W.: Proc. Soc. exper. Biol. a. Med. **31**, 864 (1934). — SCOTT, J.: Anat. Rec. **81**, 90 (1941). — Zoologica **29**, 49 (1944). — TURNER, C. L: Biol. Bull. **80**, 371 (1941). — J. exper. Zool. **91**, 167 (1942). — WITSCHI, E.: Sex and internal Secretions. Baltimore: William and Wilkins 1939. — Symp. Quant. Biol. Cold Spring Harbor **10**, 145 (1942). — WITSCHI, E. a. E. W. CROWN: Anat. Rec. **70**, 121 (1937). — WUNDER, R. W.: Z. vgl. Physiol. **63**, 596 (1931).

AMYA B. KAR, Ph. D., Zoological Laboratory
35, Ballygunge Circular Road Calcutta (Indien).