Ghana J. Sci. **29-30,** 7-11

PRELIMINARY OBSERVATIONS ON METHALLIBURE-TREATED FRY OF *SAROTHERODON NILOTICUS* DURING GONADAL DIFFERENTIATION PHASE

K. N. EYESON
Department of Zoology, University of Cape Coast, Cape Coast, Ghana

Summary

Growth and gonadal development in sexually undifferentiated *Sarotherodon niloticus* fry appeared to have been reduced by methallibure treatment administered daily at 1 and 100 mg per kg of feed for 3 weeks. The drug had no marked effect on the histology of the pituitary gland. In the distal lobe of the gland only the presumed prolactin cells and somatotropes had developed during the observation period. There was no evidence of pituitary involvement in gonadal differentiation in the fry.

Introduction

The antigonadotropic effect of methallibure (I.C.I. 33, 828) has been demonstrated in a variety of teleosts (Hoar *et al.*, 1967; Wiebe, 1968; Leatherland, 1969; Martin & Bromage, 1970; Pandey, 1970; Pandey & Leatherland, 1970; Dadzie, 1972; Hyder, 1972; Mackey, 1973; Breton, Jalabert & Billard, 1973; Van Reo, Goverdinal & Van Oordt, 1976; Singh, Raizada & Singh, 1977; Lanzing, 1978; Chiba, Honma & Lanzing, 1978).. The drug has been used mainly as a substitute for hypophysectomy in the study of the pituitary-gonadal relationships in fishes. These investigations have confirmed the pituitary control of gametogenesis and vitellogenesis. Furthermore, Latey & Rangneker (1978) have also reported that the drug suppresses androgen biosynthesis and subsequently causes the disappearance of reproductive and agonistic behaviour.

The possible use of methallibure to regulate reproduction in tilapia culture has been discussed by Dadzie (1974). Unwanted reproduction in the fish can also be prevented by using hormone treatments of the fry to produce monosex populations (Shelton, Hopkins & Jensen, 1978). Treatments with oestrogens have been less successful than with androgens (Tayamen & Shelton, 1978). In an attempt to produce oestrogen sex-reversed *Tilapia aurea (Sarotherodon aureus)* for breeding programme, Hopkins, Shelton & Engle (1979) noticed that hormone treatments combined with methallibure were the most successful.

The implication of this observation is that while methallibure, by itself, would not induce sex reversal it enhanced hormone-induced sex-reversal probably by interfering with the natural gonadal differentiation process. This could possibly be achieved either by blocking the production of pituitary gonadotrophic hormones, if these were involved, or by directly interfering with the biochemical processes in the differentiating gonad. The use of the drug alone or in combination with oestrogen to influence gonadal differentiation in other sex-reversal programmes has, however, not yielded unequivocal results (Shelton, personal communication).

The investigations on *Sarotherodon niloticus* reported here were an attempt to determine the possible role of the pituitary in gonadal differentiation in tilapia using methallibure as the antigonadotropic agent. This, it was hoped, would clarify the role of the drug in the improved oestrogen induced sex-reversal programmes reported above.

Experimental

For this work done in the Department of Fisheries, Auburn University (U.S.A.), fry of *S. niloticus* were obtained in August 1981 from brood stock kept in outdoor concrete tanks. They were transported to the laboratory and those fry measuring 9-12 mm total length were selected

and randomly sorted out into 6 groups of 100 fry each. Fish of this size range were presumed to be sexually undifferentiated (Shelton *et al.*, 1978). Three experimental groups, of two replicates each, were set up in three stainless steel tanks each measuring 210 × 30 × 20 cm deep. The replicates were separated by a fine mesh screen. The depth of water was kept at 18 cm and continuously aerated. A flow of filtered water was maintained through the system. The average water temperature was 26 °C (range 25-27 °C).

The control feed was prepared by the alcohol evaporation method as outlined by Shelton *et al.* (1978) but no hormone was used. A stock solution of methallibure was prepared by dissolving 1g in 1*l* of 95 per cent ethanol and the appropriate volumes were mixed with ground basic diet to give concentrations of 1 mg and 100 mg/kg of feed, the latter being the effective concentration used by Hopkins *et al.* (1979).

The three experimental groups of fish were separately fed on the different treatment diets at 12 per cent of their body weight per day and this was adjusted weekly. Each day's ration was given in three parts at 08.00, 12.00 and 16.00 h, using feeding rings; the tanks were cleaned three times a day to remove waste material, sediment and uneaten food. The experiment was conducted for 3 weeks and total lengths of samples of 10 fry from each replicate were measured each week.

The heads and trunks of 5 specimens of each weekly sample were fixed in Bouin's solution, dehydrated and embedded in paraffin. They were sectioned at 7μm and suitable sections of the pituitary glands and gonads were selected for staining. For the pituitary glands the trichrome stain of Cleveland and Wolfe was used but the gonads were stained with haematoxylin and eosin.

Since there was no significant difference between the replicates of each experimental group the weekly sub-samples were pooled for each treatment. Thus, weekly measurements of 20 fry were used in determining the growth rates.

Growth rates

Table 1 shows the growth rates of the fry at a stocking density of about 176 per 100 l. There was no appreciable difference in the average size of fry at the different dosage levels of the drug, but these treated fish showed a slightly reduced growth rate compared to the control fish after 3 weeks experimental period. The size range of ME-100 treated fry at 3 weeks was comparatively

TABLE 1

Weekly changes in total length of fry of S. niloticus

Treatment	*Sample size*	*Average length (mm) ± S. E. after*		
		1 week	*2 weeks*	*3 weeks*
Control	20	15.2 ± 0.3 (14 - 17*)	23.6 ± 0.3 (21 - 26)	31.9 ± 0.5 (28 - 38)
ME - 1**	20	14.3 ± 0.4 (13 - 17)	21.8 ± 0.3 (19 - 26)	29.4 ± 0.6 (25 - 35***)
ME - 100**	20	15.1 ± 0.2 (14 - 16)	22.2 ± 0.2 (21 - 26)	29.5 ± 0.4 (26 - 32***)

* Figures in parenthesis represent range values

** ME - 1 and ME - 100 represent methallibure dosage levels in mg kg^{-1} diet

*** Difference from control is significant ("t" test; $P < 0.001$)

small, probably due to the relatively high concentration of the drug. No data are available on the effect of methallibure on growth of fry, but Dadzie (1974) reported an improved growth rate of treated matured tilapia after the drug had been withdrawn.

Pituitary gland

In the pituitary gland of both the control and treated fry only the presumed prolactin cells and somatotropes had developed in the rostral and proximal pars distalis respectively. The prolactin cells were more prominently developed than the somatotropes in fry measuring 23 to 38 mm. The early development and apparently active state of the prolactin cells in the fry could be related to osmoregulatory functions under the freshwater conditions of the aquarium.

A similar activity has been reported in the hatching of the rainbow trout (Nozaki, Tatsumi & Ichikawa, 1974). Methallibure had no marked effect on the somatotropes as could be expected from its apparent inhibitory effect on growth. This could be due to the short treatment period. Perhaps some changes might be observed at the ultrastructural level. Chiba *et al.* (1978), however, noticed an inhibitory effect of the drug on the somatotropes of juvenile *Tilapia mossambica* after 7 weeks treatment, but no measurement of the fish were taken.

A possible interference of thyroid activity by methallibure has been reported in some teleosts (Leatherland, 1969; Pandey & Leatherland, 1970; Mackay, 1971) and this might have some effect on growth. However, this could not be ascertained from the present study because the thyrotropes had not differentiated in the pituitary glands of the samples examined. Unlike the other investigators mentioned above, Chiba *et al.* (1978) did not notice any appreciable change in the thyrotropes of the tilapia they studied.

Gonadal differentiation

The observation period for the present investigation was restricted to 3 weeks, the minimum duration which hormonally induced sex reversal

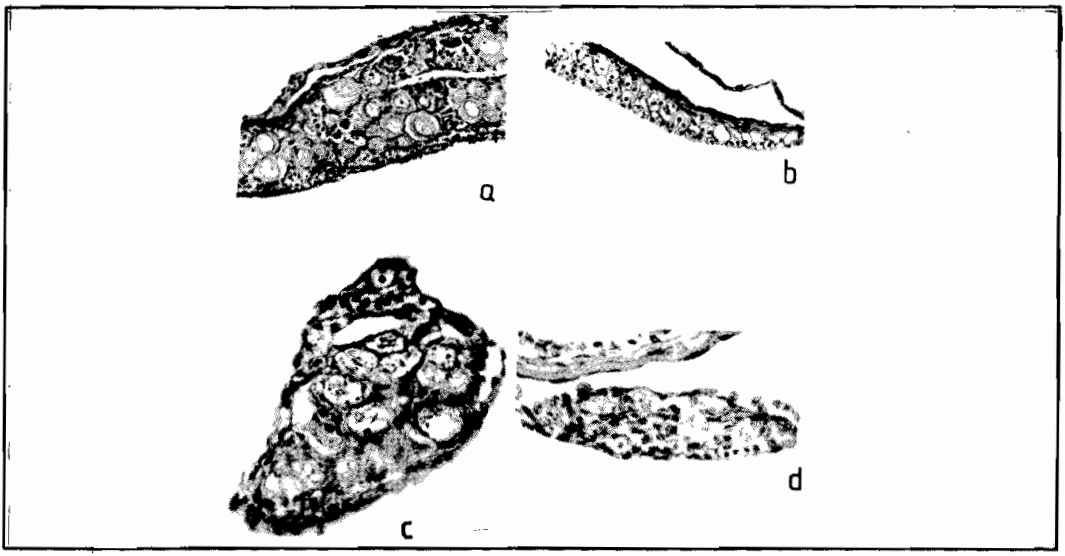

Fig. 1. Sections of gonads during 3 weeks experimental period: (a) Longitudinal section of ovary (control) showing oocytes in protoplasmic phase (x230); (b) Longitudinal section of testes (control) showing resting spermatogonia after mitotic division (x230); (c) Transverse section of ovary of ME-100 treated fish, showing chromosomal activity in oocytes before protoplasmic phase (x460); (d) Longitudinal section of testis of ME-100 treated fish showing spermatogonia before mitotic division (x510).

has been achieved (Tayamen & Shelton, 1978; Shelton *et al.*, 1981). Gonadal differentiation occurred in all treated specimens during the observation period (Fig. 1). At the end of this period more advanced stages of differentiation were found in the control fish where some of the ovaries contained oocytes in the protoplasmic phase (Fig. 1a); the most advanced testes contained spermatogonia with inactive nuclei (Fig. 1b). Since the gonadotropes had not developed in the pituitary gland of these fry it could be inferred that pituitary gonadotrophic hormones were not involved in these early stages of gonadal differentiation. However, such advanced stages of gonadal development of fishes as vitellogenesis and spermatogenesis are known to be dependent on gonadotrophic hormones. The absence of these stages in the experimental fry was therefore due to the absence of gonadotropes rather than the inhibitory effect of methallibure.

The slight difference in the rate of gonadal differentiation between the control and treated fish could be attributed to their size difference. Since growth appeared faster in the control samples, gonadal development could be more advanced than in the treated fry. Length of fry has been considered an important guide in determining the stages in gonadal differentiation in *Tilapia aurea* (Dutta, 1979).

Conclusion

Since gonadotropes were absent in the pituitary gland of fry undergoing gonadal differentiation, and methallibure appeared to have only a slight effect on the differentiating gonads, it is difficult to assess the role of this compound in the oestrogen induced sex-reversal programmes of *S. aureus* reported above. However, the drug appeared to inhibit growth. How this could enhance the efficacy of oestrogen treatment is not clearly understood. It is also not easy to explain why ME - 1 and ME - 100 produced similar results while in the experiments of Hopkins *et al.* (1979) ME - 100 proved more potent than ME - 1 in improving oestrogen sex-reversal. Further investigation may be necessary to clarify the role of methallibure in sex reversal programmes.

Acknowledgement

The author would like to express his sincere gratitude to the Department of Fisheries and Aquaculture, Auburn University, Alabama, USA, where he undertook his study on a Fulbright research fellowship, for the facilities placed at his disposal, and to Dr William S. Shelton for his assistance during the study. The fellowship is gratefully acknowledged.

References

Breton, B., Jalabert, B. & Billard, R. (1973) Pituitary and plasma gonadotrophic levels and spermatogenesis in the goldfish *Carassius auratus* after methallibure treatment. *J. Endocrinol.* **59**, 415-420.

Chiba, A., Honma, Y. & Lanzing, W. J. R. (1978) Effect of methallibure on the hypophysis and gonadal development of the cichlid fish, *Tilapia mossambica*. *Jap. J. Ichthyol.* **25**, 107 - 114.

Dadzie, S. (1972) Inhibition of pituitary gonadotrophic activity in cichlid fishes of the genus *Tilapia* by a dithiocarbamoylhydrazine derivative (I.C.I. 33, 828). *Ghana J. Sci.* **12** (2), 41-53.

Dadzie, S. (1974) A preliminary report on the use of methallibure in *Tilapia* culture. *Afr. J. Trop. Hydrobiol. Fish.* **4** (1), 127-141.

Dutta, O. K. (1979) Factors influencing gonadal sex differentiation in *Tilapia aurea* (Steindachner). (Ph.D. Dissertation),Auburn University, Auburn, AL., U.S.A.

Hoar, W. S., Wiebe, J. & Waie. H. (1967) Inhibition of the pituitary gonadotropic activity of fishes by a dithiocarbamoylhydrazine derivative (I.C.I. 33, 828). *Gen Comp. Endocrinol.* **8**, 101-109.

Hopkins, K. D., Shelton, W. L. & Engle, C. R. (1979) Estrogen sex reversal of *Tilapia aurea*. *Aquaculture* **18**, 263-268.

Hyder, M. (1972) Endocrine regulation of reproduction in *Tilapia*. *Gen. Comp. Endocrinol. Suppl.* **3**, 729-740.

Lanzing, W. J. R. (1978) Effect of methallibure on gonad development and carotenoid content of the fins of the fish *Tilapia mossambica*. *J. Fish Biol.* **12**, 181-185.

Latey, A. N. & Rangneker, P. V. (1978) Effects of methallibure on the external morphology, beha-

viour and chromatic physiology of the teleost, *Tilapia mossambica* (Peters). *Hydrobiologia* **60** (3), 221-227.

LEATHERLAND, J. F. (1969) Studies on the structure and ultrastructure of the intact and "methallibure"-treated mesoadenohypophsis of the viviparous teleost *Cymatogaster aggregata* Gibbons. *Z. Zellforsch.* **98**, 122-134.

MACKAY, N. J. (1971) The effects of methallibure (I.C.I. 33, 828) and thiourea on gametogenesis in the firetail gudgeon, *Hypseleotris galii. Gen. Comp. Endocrinol.* **20**, 221-235.

MARTIN, P. & BROMAGE, N. R. (1970) The effect of methallibure on spermatogenesis in *Peocilia reticulata. J. Fish Biol.* **2**, 47-51.

NOZAKI, M., TATSUMI, Y. & ICHIKAWA, T. (1974) Histological changes in the prolactin cells of the rainbow trout *Salmo gairdnerii irideus* at the time of hatching. *Annotnes zool. jap.* **47**, 15-21. Cited in *Comparative Endocrinology of Prolactin* by ENSOR, D. M. (1978). Chapman and Hall, London, 309 pp.

PANDEY, S. (1970) Effects of methallibure on the testes and secondary sex characters of the adult and juvenile guppy *Poecilia reticulata* Peters. *Biol. Reprod.* **2**, 239-244.

PANDEY, S. & LETHERLAND, J. F. (1970) Comparison of the effects of methallibure and thiourea on the testis, thyroid, and adenohypophsis of the adult and juvenile guppy, *Poecilia reticulata* Peters. *Canad. J. Zool.* **48**, 445-450.

SHELTON, W. L., HOPKINS, K. D. & JENSEN, G. L. (1978) Use of hormones to produce monosex tilapia for aquaculture. In R. O. Smitherman, W. L. Shelton & J. H. Grover (Ed.), *Proceedings Culture of Exotic fishes Symposium* Fish Culture Section, AM Fish. Soc., Auburn University, Auburn, AL, pp. 10-33.

SINGH, T. P., RAIZADA, R. B. & SINGH, A. K. (1977) Effect of methallibure on gonadotrophic content, ovarian 32p uptake and gonadosomatic index in freshwater catfish, *Heteropneustes fossilis* (Bloch). *J. Endocrinol.* **72**, 321-327.

TAYAMEN, M. M. & SHELTON, W. L. (1978) Inducement of sex reversal in *Sarotherodon niloticus* (Linnaeus). *Aquaculture.* **14** (4), 349-354.

VAN REO, GOVERDINAL, E. & VAN OORDT, P. G. W. (1976) Effects of methallibure (I.C.I. 33, 828) and ovine luteinizing hormones on the pituitary of the female zebrafish, *Brachydanio rerio.* (Abstracts, 8th Conference of European Comparative Endocrinologists, Aug. 11-16, 1975, Bangor, Gt. Britain.) *Gen. Comp. Endocrinol.* **29**, 263.

WIEBE, J. P. (1968) Inhibition of pituitary gonadotropic activity in the viviparous seaperch, *Cymatogaster aggregata* Gibbons by a dithiocarbamoylhydrazine derivative (I.C.I. 33, 828). *Canad. J. Zool.* **46**, 751-758.

Received 7 Aug 86; revised 12 Apr 89.