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The effect of methallibure (I.C.I. 33, 828) on the steroidogenesis in the ovary and testis of a fresh water teleost, *Cyprinus carpio*

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Summary. Methallibure treatment of mature *Cyprinus carpio* causes a marked reduction in the steroidogenesis, as indicated by the fall in 3β -HSD activity in the gonads. Significance of these results is discussed.

The surgical hypophysectomy and replacement therapy remain the most convincing experimental procedures for the demonstration of pituitary regulation of gonadal functions. Hypophysectomy is not only technically difficult, but it also disrupts the entire endocrine system. For the specific gonadotrophin suppression, several compounds have been used, most of which are steroids which invariably interfere with feedback pathways². In recent times several workers have advocated the use of a nonsteroidal antigonadotrophic compound (methallibure; I.C.I. 33, 828) in place of surgical hypophysectomy³⁻¹². Wiebe⁴ and Van Ree¹⁰ reported a reduction in the level of histochemically demonstrable steroid dehydrogenases in the gonads as a result of methallibure treatment. The present study deals with the effect of methallibure treatment on the activity of A_5 - 3β -hydroxysteroid dehydrogenase (3β -HSD) in the gonads of fish, *Cyprinus carpio*.

66 mature specimens (33 female and 33 male) of *Cyprinus carpio* measuring 25-30 cm and weighing 100-120 g, were divided into 11 separate groups (each group consisting of 3 female and 3 male specimens), and housed in 300 l aquaria, which were kept aerated periodically. No feeding was done during the experimental period, which lasted 35 days. A suspension containing 1.0 g methallibure/100 ml distilled water was prepared and maintained with Tween 80 (2 drops/10 ml). Groups 1-5 were given 3.0 ml dose, and groups 6-10, 5.0 ml dose, on alternate days. The suspension was dissolved in the aquarium water. The experiments were timed in such a way that 10th day of groups 1 and 6, 20th day of groups 2 and 7, 25th day of groups 3 and 8, 30th day of groups 4 and 9, and 35th day of groups 5 and 10, fell on the same day. Group 11 served as control. The procedure regarding the determination of enzyme activity has already been reported¹³. The only modification made was that, instead of the mixture of propylene glycol and dimethyl formamide (1:1), the dehydroepiandrosterone (DHA) was dissolved in dimethyl formamide. The relative differences in the enzyme activity between the control (group 11) and various experimental groups (1-10), and among the various experimental groups (1-5, and 6-10), were worked out and

the Student t-test was applied to the data for comparing the effect of one exposure period and/or the concentration of the chemical, with the others.

Results and discussion. The table summarizes the results of the experiments. It can be seen that methallibure treatment caused a time-dependent reduction in the activity of 3β -HSD, both in the ovary and the testis of *Cyprinus carpio*. The differences with regard to the enzyme activity between the control and various treated groups (1-10) are highly significant ($p < 1\%$). There is also a significant difference ($p < 5\%$) in the enzyme activity among the different groups (1-5), and (6-10). The differences within each group were insignificant. Also, when the enzyme activity between the 2 groups treated for the same period was compared with regard to the effect of concentration of methallibure, the difference were found to be statistically insignificant. This may probably indicate (though within a limited range of dosages used) that the time interval may be critical for the proper effect of methallibure on the steroidogenesis in fish gonads.

It may be mentioned that the differences in enzyme activity between the groups exposed to 35 day and 40 day treatment (at both concentrations) were insignificant and have not been recorded in the table.

The level of 3β -HSD, along with that of various other steroid dehydrogenases, is indicative of steroidogenesis in the gonadal tissue (see ref. 14 for various criteria of steroidogenesis in a tissue). Hypophysectomy in animals causes a reduction in the histochemically demonstrable level of 3β -HSD¹⁵. The fall in the histochemically demonstrable^{4,10} and biochemically demonstrable level of 3β -HSD (as observed during the present studies), may be because of the action of methallibure at gonadotrophin level^{4,10,12}. The exogenous gonadotrophin treatment in case of methallibure-treated fish/hypophysectomized animals, stimulates the activity of steroids dehydrogenases¹⁶⁻²⁰. The gonadotrophins are also known to stimulate cyclic AMP synthesis²¹⁻²⁴ (via adenyl cyclase stimulation)²¹, and also RNA and protein synthesis²⁵⁻³⁰. The action of methallibure, as related to the steroidogenesis in the gonadal tissue, may

The effect of methallibure on the activity* of 3 β -HSD in the ovary and testis of *Cyprinus carpio*

Sex	Activity of 3 β -HSD (units)**		After an exposure period (days) to methallibure				
	Control		10	20	25	30	35
Female	11.15 \pm 1.45	Lower dose	8.79 \pm 0.91	7.63 \pm 0.50	3.59 \pm 0.27	2.20 \pm 0.07	1.11 \pm 0.11
		Higher dose	7.07 \pm 0.49	6.59 \pm 0.33	3.23 \pm 0.55	1.91 \pm 0.22	1.03 \pm 0.10
Male	6.8 \pm 0.66	Lower dose	5.77 \pm 0.36	4.76 \pm 0.28	3.29 \pm 0.29	1.83 \pm 0.12	1.09 \pm 0.14
		Higher dose	5.33 \pm 0.65	4.37 \pm 0.23	2.93 \pm 0.36	1.45 \pm 0.18	0.93 \pm 0.08

* The results are mean values with SE, for 3 animals; ** the activity of enzyme is in terms of number of units per mg protein, where one unit is equivalent to change in an OD of 0.01/min.

therefore be either by inhibition of gonadotrophin synthesis/release by the pituitary, or by interference with adenyl cyclase-AMP-protein kinase, system/ stimulation of the activity of phosphodiesterase or the inhibition of RNA/protein synthesis. However, much work needs to be done before we can reach any conclusion about the mode of action of methallibure at the molecular/physiological level.

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Ergastoplasmic granules, cytophysiological adaptation of the locusts corpora cardiaca to migratory flights?

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Summary. A good correlation was shown between the presence of ergastoplasmic granules in the glandular cells of the locusts corpora cardiaca and the flight activity of these insects.

Some species of locusts are able to accomplish sustained migration flights. Lipids and carbohydrates are the fuels mobilized in the fat body by means of a metabolic hormone synthesized in the glandular lobes of the corpora cardiaca (CCG)²⁻⁵. The secretory cells contain a lot of dense, circular and smooth membrane-bounded granules originating in the Golgi cisterns (diameter up to 600 nm); these classical granules may be associated with dense, ergastoplasm-bounded, circular grains (EG) (diameter up to 5 μ m)⁶ and their significance is here investigated.

Male adults of *Schistocerca gregaria* Forsk. were kept under standard grouped conditions⁷; their flying capacity was determined⁸ and the 'good fliers' only were chosen. Roundabouts were used in the flight experiments⁹. The CCG were fixed with glutaraldehyde and postfixed by OsO₄ (2% solutions in Na-cacodylate buffer 0.1 M, pH 7.4, containing 8% of sucrose) and embedded in a mixture of epon-araldite. Semi-thin sections were stained with 0.5%

toluidine blue in 1% borate. Ultra-thin sections were contrasted with uranyl acetate and lead citrate.

5 experimental groups were compared: 2 groups of controls (not subjected to flight) which are directly used without pretreatment at the age of 20 days (T1) or of 40 days (T2); 2 groups of 40-day-old animals which have flown before fixation for 3 h (short flight: V3) or for 20 h (long flight: V20); 1 group of locusts having flown at the age of 20 days were fixed after a 20-day-rest period (VR). The number of only the big EG (diameter from 1.5 μ m) per 600 μ m² tissue is calculated on semi-thin sections at the magnification of \times 960; at least 50.000 μ m² tissue per animals was investigated. The results were analyzed with the t-test and are presented in the table and the figure.

EG were found in the CCG of all controls, more so in 40-day- than in 20-day-old animals. After a 3 h flight (V3) only half of the examined CCG still contained EG, whose number and volume were significantly reduced