

rabbit there are correlated changes in the levels of progesterone and protein in the uterine flushings during early pregnancy and pseudopregnancy, though the biological significance of these changes has not yet been demonstrated<sup>20</sup>. The total amounts of uterine luminal progesterone on day 4 of pseudopregnancy were low but similar in young and aged mice (table 2). These progesterone levels were not standardized for uterine weight, even though old uteri were heavier than young ones ( $p < 0.001$ ), because there was no evidence of an association between increased uterine weight and glandular surface area. The levels of protein in uterine flushings of individual mice were also low and below the sensitivity of the technique (table 2), although Aitken<sup>21</sup> has demonstrated a small transient increase in the protein content of pooled samples of uterine flushings on day 5, which is the day on which implantation occurs in pregnant animals. If the relationship between levels of progesterone and of protein holds in the mouse as it does in the rabbit, the results of table 2 imply that the amounts of uterine protein are unaffected by ageing of the uterus.

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## The effect of gonadotrophins on the steroidogenesis, in the ovary and testis of gonadotrophin-deprived fresh water teleost, *Cyprinus carpio*

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**Summary.** Studies have been made on the effect of LH, FSH, and LH + FSH on the gonadal steroidogenesis in gonadotrophin-deprived common carp. LH alone and in combination with FSH was more effective than FSH in stimulation of steroidogenesis.

The surgical hypophysectomy followed by replacement therapy has been the most convincing experimental method for the demonstration of pituitary control of gonadal functions. Since the hypophysectomy disrupts the endocrine balance, several chemical compounds, most of which are steroids, have been used for specific gonadotrophic suppression. These steroids invariably interfere with the feedback pathways<sup>2</sup>. In recent times, several workers have advocated the use of a nonsteroidal antigonadotrophic compound (methallibure, ICI 33,828) in place of surgical hypophysectomy<sup>3-15</sup>. The gonadotrophic inhibitory properties of this compound have been substantiated by studies showing arrest of gametogenesis<sup>3,4,8,11,13</sup>, inhibition of development of secondary sexual characters<sup>3,8</sup>, histological and cytological changes in the pituitary gonadotrophs<sup>5,9,15,16</sup> plasma gonadotrophin level<sup>10</sup>, <sup>32</sup>P uptake by ovary<sup>14</sup>, and histochemical<sup>4,13</sup> and biochemical<sup>17</sup> activity of steroid dehydrogenases.

The level of  $\Delta 5\text{-}3\beta\text{-hydroxysteroid dehydrogenase}$  ( $3\beta\text{-HSD}$ ), along with that of various other steroid dehydrogenases, is indicative of steroidogenesis in the gonadal tissue<sup>18</sup>. During the present studies, investigations have been made on the effect of LH, FSH and LH + FSH, on the steroidogenesis (with regard to the activity of  $3\beta\text{-HSD}$ ) in the ovary and testis of gonadotrophin-deprived teleost fish, *Cyprinus carpio*.

**Materials and methods.** 30 mature specimens (sex ratio 1:1), of *Cyprinus carpio* measuring 25–30 cm and weighing 100–120 g, were divided into 5 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. Groups 2–5 were treated with methallibure as described earlier<sup>17</sup>. The gonadotrophin treatment consisted of 5 i.p. injections (on alternate days), of 7.5  $\mu\text{g/g}$  each of bovine FSH and LH to groups 3 and 5, respectively, and FSH + LH (7.5  $\mu\text{g/g}$  each), to group 4. Group 1 served as untreated control and group 2, as a control for the gonadotrophin treated groups. Both of these groups were given 5 injections of 0.9% saline (0.2 ml in each injection), at the same time when the gonadotrophin treatment (dissolved in 0.2 ml of 0.9% saline for each injection), was being given to other groups. The procedure regarding the determination of enzyme activity has been reported earlier<sup>17,19</sup>. The relative differences in the enzyme activity between the untreated control (group 1) and various experimental groups (2–5), and among the various experimental groups (2–5), were worked out and Student's t-test was applied to the data for making comparison of the effect of gonadotrophin-deprivation and various gonadotrophin-treatments with respect to enzyme activity.

**Results.** Table 1 summarizes the results of the experiments.

The effect of LH, FSH and LH + FSH on the activity \* of  $3\beta$ -HSD in the ovary and testis of gonadotrophin-deprived *Cyprinus carpio*

Sex	Activity ** of $3\beta$ -hydroxysteroid dehydrogenase				
	Untreated controls	Methallibure treated groups Control	LH treated	LH + FSH treated	FSH treated
Female	11.15 $\pm$ 1.45	1.03 $\pm$ 0.10	9.85 $\pm$ 0.14	10.71 $\pm$ 0.41	6.53 $\pm$ 0.41
Male	6.80 $\pm$ 0.66	0.93 $\pm$ 0.08	6.03 $\pm$ 0.30	6.23 $\pm$ 0.19	4.37 $\pm$ 0.32

\* 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 1 unit is equivalent to change in an OD of 0.01 per min.

It can be seen from the table that methallibure treatment for 35 days caused a highly significant reduction in the activity of  $3\beta$ -HSD, both in the ovary and the testis of *Cyprinus carpio*. The treatments by LH alone and LH+FSH in combination, stimulated the  $3\beta$ -HSD activity in the gonads and brought it to the level of untreated controls. The differences in the enzyme activity among the untreated controls, LH treated and LH+FSH treated groups have been found to be insignificant ( $p < 5\%$ ). The differences in the enzyme activity between the above-mentioned groups and the FSH treated groups, and also between the FSH treated groups and the methallibure treated control, are significant ( $p < 1\%$ ).

**Discussion.** Witschi<sup>20</sup> and Otsuka<sup>21</sup> suggested that pituitary of *Oncorhynchus* contains both LH and FSH activities. Several studies indicating the high specificity of glycoprotein pituitary hormones<sup>22-24</sup> have cast doubts on the results of Witschi<sup>20</sup> and Otsuka<sup>21</sup>, who had used non-teleostean bioassays. Several studies have lead to the isolation of single gonadotrophic factor capable of restoring the various maturation phases of gonads and their release<sup>7,23-29</sup>. Recently Haider and Blum<sup>30</sup> obtained 2 fractions from goldfish pituitary, one inducing spermatogenesis only, and the other, inducing spermatogenesis, spermination and activation of interstitial cells in case of *Xiphophorus*.

Studies on the effect of exogenous mammalian hormones on various aspects of teleost reproduction indicate that LH

or LH-like preparations are active, whereas FSH is inactive<sup>31-37</sup>. Singh<sup>38</sup>, on the other hand, claimed that mammalian FSH was more potent than LH and HCG in promoting increase in ovarian weight in hypophysectomized *Mystus vittatus*. Hormonal treatment was thought to cause only the gonadal hydration in this case, and the gonadotrophins used were possibly contaminated<sup>39</sup>. Dadzie<sup>40</sup> reported that in case of methallibure-treated *Tilapia*, a combination of LH and FSH, when given consistently, stimulated the regressed gonads to spawning condition; LH and FSH, when used separately, were less effective. Of these 2 hormones, LH was more effective.

During the present studies, FSH, LH and a combination of FSH+LH caused a marked increase in the gonadal level of  $3\beta$ -HSD, as compared to methallibure-treated controls. Insignificant differences existed in the enzyme activity between the LH, and LH+FSH-treated groups, whereas these groups greatly differed in  $3\beta$ -HSD activity from the FSH-treated groups. The conflicting results obtained by several workers may indicate a great variation among the teleost fishes in responsiveness to mammalian gonadotrophins owing to the species specificity<sup>22-24</sup>, or possibly due to the differences in the ability of the receptors at target structures to bind the gonadotrophins<sup>41</sup>. Much work, therefore, needs to be done before the controversy regarding the nature of piscine gonadotrophins on various aspects of teleost reproduction may be finally solved.

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