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Effects of triiodothyronine and of some gonadotropic and steroid hormones on the maturation of carp (*Cyprinus carpio* L.) oocytes *in vitro*

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Summary. The effects of triiodothyronine (T_3) and of gonadotropic and steroid hormones on carp oocyte maturation *in vitro* were investigated using ovarian fragments from 5 females that had completed vitellogenesis.

The percentages of mature oocytes were consistently greater in the subgroups incubated with T_3 + steroid hormone, or with T_3 + gonadotropic hormone, than in the subgroups incubated with the same steroid or preparation of gonadotropic hormone without T_3. Differences between the first and second groups proved to be statistically significant ($P < 0.01$). The results suggest that T_3 influenced the maturational response of carp oocytes to some gonadotropic and steroid hormones.

Introduction.

Many data show that thyroid hormone plays a role in fish reproduction, although its mechanism is not clear (Fontaine, 1976). Very intense activity of the thyroid gland was observed histologically in male salmon species during the spawning period (Barannikova, 1978). In the females of this species, high gonadotroph activity was accompanied by simultaneous and very intense thyrotroph activity (Barannikova, 1978). Intramuscular injections of testosterone propionate in immature trout cause both an acceleration of thyrozine deiodization and an increase in the triiodothyronine (T_3) level (Hunt and Eoles, 1976).

Blockade of thyroid gland activity by goitrogenesis or destruction of the gland by radioiodine inhibits gonadal development in some teleost fish species (Fontaine, 1976). An investigation by Dettlaff and Davydova (1979) has shown that T_3 restores gonadotropic sensitivity of the follicular cells in sturgeons kept at too low a temperature or in unsuitable conditions. The participation of thyroid hormones in the gonadal development of goldfish was described in a review by

Lam *et al.* (1978) who found that these hormones act in synergy with gonadotropin on vitellogenesis and on the maintenance of the oocytes after completion of vitellogenesis. Sage and Bromage (1970) and Sage and Berin (1971) have shown that the gonadotropic cells at the pituitary level in *Poecilia reticulata* are inhibited by both androgens and estrogens *in vitro* but that *in vivo* estrogens stimulate the TSH cells. Injections of 17β-estradiol make the thyrotrophs of the European eel more active (Olivereau, 1979). The aim of the present work was to determine if the addition of T_3 to the incubation medium would influence the maturational response of carp oocytes to gonadotropin or selected steroid hormones (Epler, 1981a, b, c).

Material and methods.

Investigations were carried out in April using ovarian fragments from 5 adult female carp from commercial carp ponds and weighing 4.5 to 5.0 kg. Twenty-four hours before taking these ovarian samples, the females were injected with carp hypophysial homogenate (chh) at a dose of 0.5 mg/kg body weight. The oocytes were incubated for 72 h in vials with 2 ml of basic salt solution (BSS), Cortland medium, at pH 7,6 and 20 $\pm$ 1 °C, with no special gas atmosphere. Each vial contained ovarian fragments with 230 $\pm$ 30 oocytes that had completed vitellogenesis. None of the oocytes had undergone maturation (germinal vesicle breakdown : GVD), although 50-60 % showed GV in the peripheral zone close to the micropyle. As indicated by the position of GV, the rest were oocytes in younger maturational stages. Ovarian fragments from all females were divided into two groups :

— group I : oocytes incubated in medium supplemented with gonadotropin or one of the steroid hormones, but without T_3 (9 subgroups, 1 control without any hormone ; fig. 1) ;

— group II : oocytes incubated in medium supplemented with gonadotropin or one of the steroid hormones and with T_3 (9 subgroups, 1 with T_3 alone ; fig. 1).

The centrifuged chh was added to the medium at a dose of 100 µg/ml. Partially purified carp gonadotropic hormone (pp c-GHT) was used at a dose of 100 ng/ml of medium. Steroids, *i.e.* testosterone (T), androsterone (A), progesterone (P), 17α-20β-progesterone (17α-20β-P), deoxycorticosterone acetate (DOCA) and cortisone acetate were used at a dose of 1 µg/ml and T_3 at a dose of 0.5 µg/ml. After incubation the oocytes were cleared in turpentine oil, and the number of mature oocytes was counted under a light microscope to calculate the percentage of maturation. Hypophyses were collected into acetone from spawners in the autumn and stored as powder. Pp c-GTH (prepared by Dr. B. Breton) and 17α-20β-P were obtained from INRA (France) ; the other steroid hormones were purchased from Merck Co. T_3 was purchased from Fluk AG Buchs SG. The results are presented at the percentage of mature oocytes with GVBD obtained for each treatment of the oocytes of each fish. The data obtained were checked by analysis of variance to determine if differences

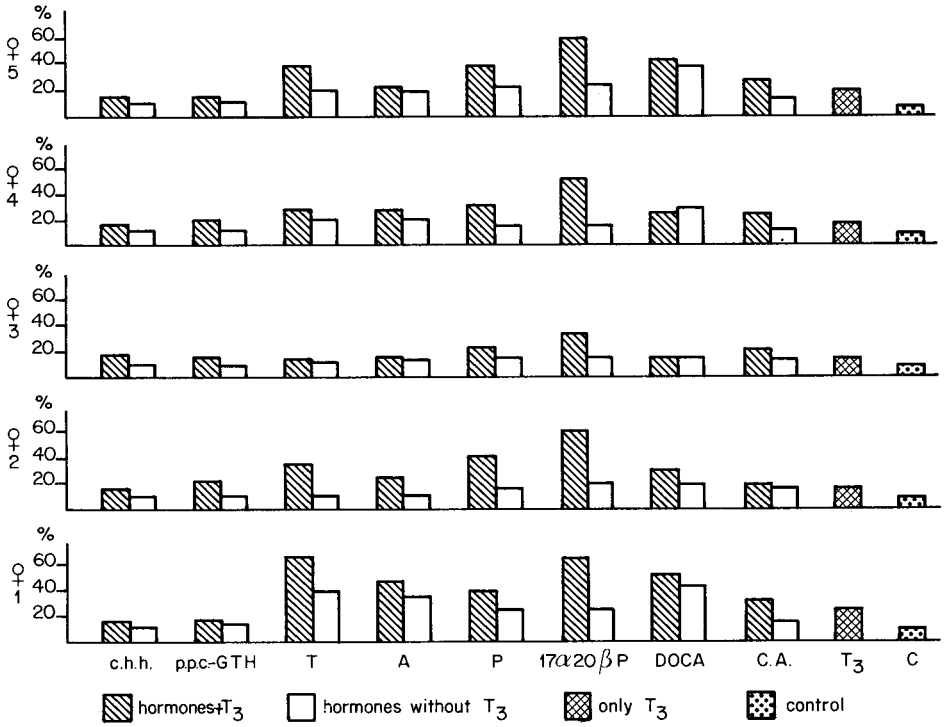

FIG. 1. — *Percentage of mature carp oocytes obtained in media with T_3 and in media without T_3.* chh : carp hypophysial homogenate ; pp c-GTH : partly purified carp gonadotropin hormone ; T : testosterone ; A : androsterone ; P : progesterone ; 17α-20β-P : 17α-20β-dihydroprogesterone ; DOCA : deoxycorticosterone acetate ; CA : cortisone acetate ; T_3 : triiodothyronine ; C : control.

between the percentage of mature oocytes in the first and second groups after 24 h of incubation were statistically significant.

Results and discussion.

The percentage of mature oocytes was higher in group II (with T_3) than in group I (without T_3) in 43 cases out of 45 studied (fig. 1, table 1). In one case it was the same (female no. 3, DOCA, fig. 1) and in the other it was a little lower (female no. 4, DOCA, fig. 1) ; the percentage of mature oocytes appeared to be more statistically significant ($P < 0.01$) in group II than in group I (table 2).

The addition of testosterone, androsterone, progesterone, 17α-20β-progesterone, DOCA cortisone acetate or T_3 alone to the medium caused a significant increase in the percentage of mature oocytes compared to the control (fig. 1, table 1). This agrees with results obtained by Epler (1981a, b, c) who found that the steroids and gonadotropic hormones used in our study stimulated carp oocytes to mature *in vitro*. The addition of T_3 alone caused about the same increase in the percentage of mature oocytes as the addition of any of the steroid

TABLE 1

Percentage of mature carp oocytes obtained in media with T_3 and in media without T_3.

Subgroup (hormone)	N° of females	Group	
		I without T_5	II with T_3
Carp hypophysial homogenate	1	12	16
	2	10	16
	3	10	18
	4	11	17
	5	11	26
	Mean	10.8	18.6
	Standard deviation	0.84	4.22
pp c-GTH	1	16	18
	2	10	22
	3	10	16
	4	12	20
	5	12	17
	Mean	12	18.6
	Standard deviation	2.45	2.41
Testosterone	1	40	68
	2	10	36
	3	12	14
	4	20	38
	5	21	40
	Mean	20.6	39.2
	Standard deviation	11.87	19.21
Androsterone	1	36	48
	2	10	26
	3	14	16
	4	20	27
	5	20	23
	Mean	21	28
	Standard deviation	9.90	11.98
Progesterone	1	26	48
	2	16	26
	3	14	16
	4	15	27
	5	22	23
	Mean	18.6	28
	Standard deviation	5.18	11.98
17β-20β-P	1	26	66
	2	20	62
	3	16	44
	4	16	52
	5	25	63
	Mean	20.6	57.6
	Standard deviation	4.77	9.15

DOCA	1	44	54
	2	20	32
	3	16	16
	4	30	27
	5	40	46
	Mean	30	25
	Standard deviation	12.16	15.13
Cortisone acetate	1	16	32
	2	18	20
	3	14	22
	4	12	20
	5	15	30
	Mean	15	26
	Standard deviation	2.24	5.10
Control	1	10	26
	2	10	18
	3	10	16
	4	10	18
	5	10	22
	Mean	10	20
	Standard deviation	0.00	4.00

TABLE 2

Analysis of variance.

	Sum of squares	Of	Mean square	F ratio
Between-group	3 559.5111	1	3 559.5111	22.7728**
Within-group	13 754.8889	88	156.3056	
Total	17 314.4000	89		

** Statistically significant ($p < 0.01$).

hormones. However, when T_3 was added to the medium with any of the steroid hormones used, or with chh and pp c-GTH, it caused an increase in the percentage of mature oocytes compared to the same steroid or pituitary hormone treatment without T_3.

The results obtained in this study demonstrate that T_3 affects the last stages of carp oocyte maturation (GVBD). These results also confirm those obtained by Hurlburt (1977), but that author worked with T_4 in goldfish *in vivo*. Hurlburt found that thyroid hormones act synergistically with gonadotropin on ovarian development in goldfish and that T_4 increases ovarian sensitivity to gonadotropic stimulation.

T_3 may play an indirect role by stimulating the metabolic processes in oocytes, thus making the oocytes more sensitive to the effect of steroids or

gonadotropic hormones. The direct effect of T_3 on carp oocyte maturation cannot be excluded either. Our results also suggest that T_3 and 17β-20α-P may work together since the combination of these gave the greatest response. If these results are confirmed by *in vivo* experiments, thet could be of importance in fishery practice.

Conclusion.

T_3 has an indirect or direct effect on the maturation of carp oocytes.

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Résumé. *Effet de la triiodothyronine, de la gonadotropine et d'hormones stéroïdiennes sur la maturation* in vitro *d'ovocytes de carpe (*Cyprinus carpio *L.).*

Les effets de la triiodothyronine, de la gonadotropine et d'hormones stéroïdiennes sur la maturation de l'ovocyte de carpe *in vitro* ont été examinés. Les fragments ovariens provenaient de 5 femelles en fin de vitellogenèse. Dans les sous-groupes où les hormones gonadotropes et stéroïdiennes sont associées à la triiodothyronine les pourcentages d'ovocytes matures sont significativement plus élevés ($P < 0,01$) que lorsque ces hormones sont utilisées seules.

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