the fact that the gamma amylase activity under starvation stress was enhanced to produce glucose directly from stored glycogen as an alternate pathway of carbohydrate metabolism.

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Thyroid hormone stimulates progesterone release from the ovary of a fish, *Anabas testudineus*

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Release of steroid hormone from the ovary is so far believed to be regulated solely by gonadotropic hormones. Thyroid hormone has been implicated in the reproduction of vertebrates but its role is rather indirect. Addition of thyroid hormone, triiodothyronine (T_3), to perch oocyte culture in vitro surprisingly resulted in a dose-dependent increase in progesterone release into the medium. To examine the specificity of this stimulation, perch were treated with thiourea, an antithyroid drug, for 7 days and oocytes from them when cultured in the presence of T_3 did not show increase of progesterone release over the control. Addition of cycloheximide with T_3 into the oocyte culture completely inhibited T_3 stimulation of progesterone release suggesting that the effect of T_3 on stimulated progesterone release is not direct and it possibly involves a protein(s) mediator. To reinforce this contention, control and T_3-treated oocytes were homogenized, fractionated and a $2 \times 10^5 g$ supernatant (200 K sup) was added to oocyte culture. 200 K significantly stimulated progesterone release and this property could be destroyed by trypsin digestion. Such an increase in progesterone release was not effected by 200 K sup from control oocytes. Results indicate that T_3-stimulated progesterone release from perch oocytes via the induction of proteinaceous factor.

STEROID hormone synthesis and release from the gonad of vertebrates including human being is known to be regulated by brain-pituitary axis. Hypothalamus of the brain releases a decapeptide hormone, gonadotropin-releasing hormone, which stimulates the release of gonadotropins from the pituitary. Gonadotropins then act on the target tissue i.e. ovary and testis, cause steroid hormones synthesis and release which in turn control the development and maturation of gonad. This is a well-established concept and till date no other hormones have been reported to have a direct effect on the gonadal steroid hormone synthesis or release. Thyroid hormone has long been implicated in the reproduction of vertebrates[1-5] and it has been shown to influence both ovarian and testicular functions in mammals[6-10]. These reports indicate an influence of thyroid hormone on gonadal activity but how it does so remains unclear. Recently we have reported thyroid hormone receptor in the nuclear preparation from perch ovary[11] and human corpus luteum[12]. These reports imply a direct influence of thyroid hormone in the reproduction. In this communication we report biological relevance of thyroid hormone receptor in the ovarian nuclei of perch.

Ovaries from the perch (*Anabas testudineus*) belonging to the prespawning stage of the annual reproductive cycle (April-May) were dissected out, oocytes isolated and cultured *in vitro* for 4 hours at 30°C by following the methods reported earlier[13]. Equal amounts of (25 mg) oocytes were placed in a sterile beaker containing ice-cold Earle's minimum essential medium (MEM) gassed with 95% O_2 and 5% CO_2. Viability of oocytes was examined by Trypan blue dye exclusion method and at the end of 4 hour more than 90% of oocytes were found viable. We have used triiodothyronine (T_3) as thyroid hormone since this is biologically more active than thyroxine (T_4). Progesterone released into the culture medium was extracted and estimated by specific progesterone radioimmunoassay. Progesterone antibody was a kind gift from Dr G. Niswinder, University of Colarado, USA.

Addition of T_3 in two different doses, i.e. 200 and

400 ng results in a dose-dependent increase of progesterone release (Figure 1). This is indeed surprising as progesterone release from the ovaries of any vertebrates is known to be controlled by gonadotropic hormones. To prove the specificity of thyroid hormone in eliciting the progesterone release from oocytes, perch were injected with thiourea (10 μg/100 g bw), an antithyroid drug, daily for 7 days. When blood was collected on the 8th day from the treated fish it showed undetectable levels of T_3 and T_4 (Table 1). Oocytes from thiourea treated and control perch were then cultured in the presence or absence of T_3 (200 ng). T_3 increased the release of progesterone almost 3-fold higher as compared to the control whereas progesterone release was significantly inhibited ($p < 0.01$) in the oocytes obtained from thiourea-treated fish. Addition of the same dose of T_3 to thiourea-treated fish oocytes did not increase progesterone release more than control oocytes (Figure 2). Results thus indicate a specific effect of thyroid hormone in the stimulation of ovarian progesterone release from perch oocytes.

As the stimulatory effect of T_3 on progesterone release from oocytes was observed in *in vitro* culture of oocytes, the question of interference by gonadotropin does not arise. However, it would be pertinent to ask how thyroid hormone can stimulate progesterone release from the oocytes. To answer this question is now difficult but it is known that binding of thyroid hormone to its nuclear receptor in the target tissue causes protein synthesis[14–15]. Moreover, we have

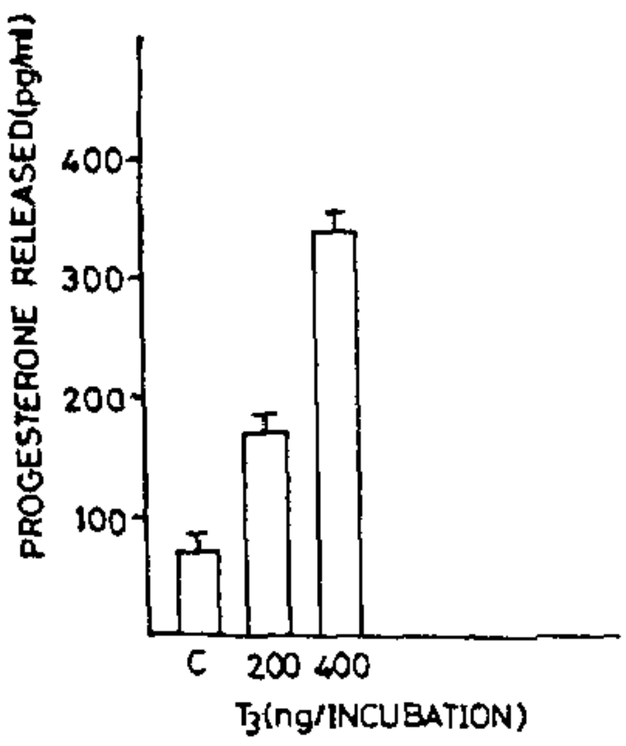

Figure 1. Effect of increasing concentrations of T_3 on progesterone release from perch oocytes. C-Control.

Table 1. T_3 and T_4 level in the serum of thiourea-treated fish

System	T_3 (ng/ml)	T_4 (ng/ml)
Saline-treated	2.2 ± 0.31	6.5 ± 0.11
Thiourea-treated	N.D.	N.D.

Sera T_3 and T_4 were determined by specific T_3 and T_4 RIAs

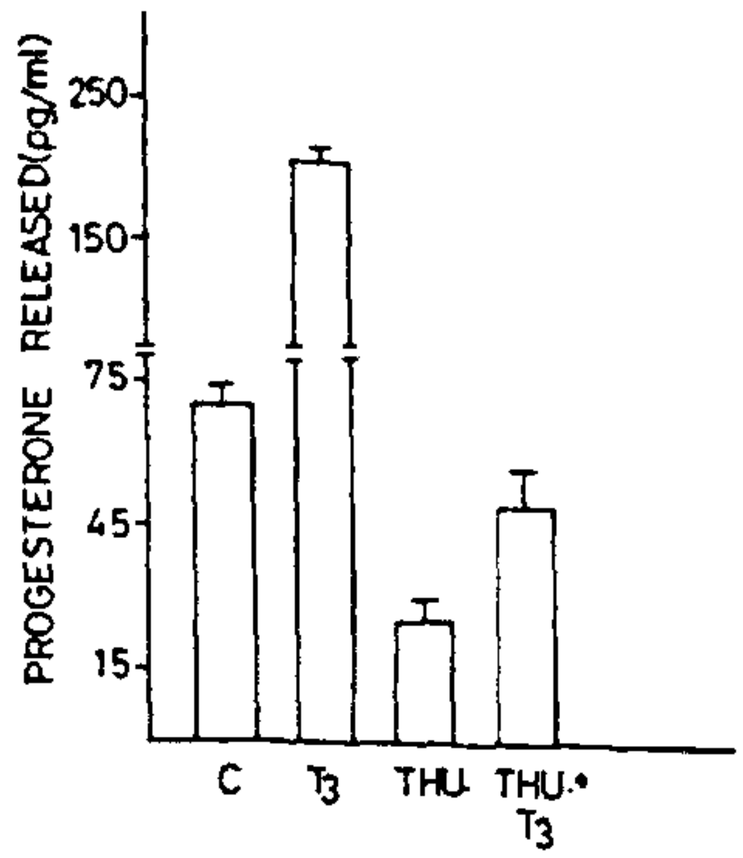

Figure 2. Inhibition of T_3 stimulated progesterone release from perch oocytes due to thiourea treatment, C-Control and THU-Thiourea.

shown earlier that binding of T_3 to perch ovarian nuclear receptor resulted in a statistically significant elevation of ovarian protein synthesis[11]. Hence, our attention was directed to the question whether stimulation of progesterone release by T_3 is a direct action of T_3 or mediated by newly synthesized protein(s). For this purpose cycloheximide, an inhibitor of protein synthesis, was added to oocyte culture with T_3. T_3 stimulation of progesterone release was completely inhibited by cycloheximide indicating that T_3-induced protein(s) in the oocytes is responsible for increased release of progesterone (Figure 3). For further evidence, both T_3-treated (200 ng) and control oocytes were homogenized and the homogenate was differentially centrifuged in a Beckman ultracentrifuge (L7–55).

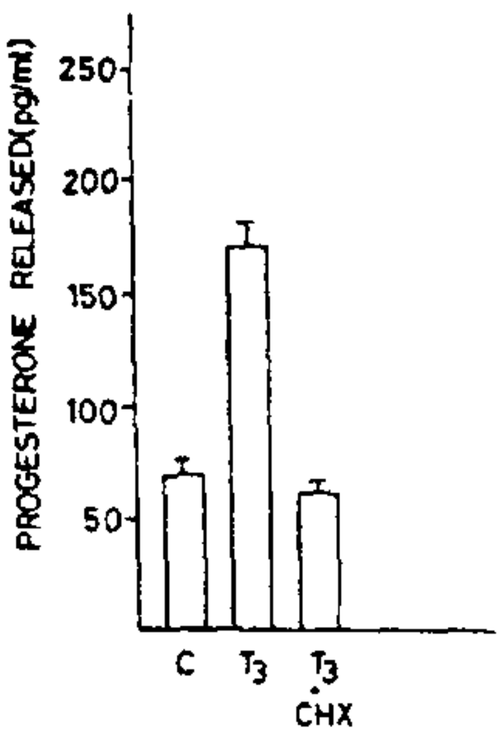

Figure 3. Cycloheximde (CHX) inhibition of increased progesterone release due to T_3 from perch oocytes. 500 μg/culture cycloheximide was added with 200 ng of T_3 or only 200 ng of T_3 or none i.e. control (C).

Table 2. Effect of 200 K supernatant fraction from T_3-treated and control oocytes on progesterone release

Treatment	Progesterone released (pg/ml)
Control oocytes (CO)	62 ± 5.3
CO + 200 K sup from control	70 ± 8.2
CO + 200 K sup from T_3 treated oocyte	140 ± 24
CO + 200 K sup from T_3 treated oocytes subjected to trypsin digestion (200 μg)	64 ± 5.1

In 200 K supernatant fraction of T_3-treated oocytes a new property was identified i.e. addition of this 200 K supernatant fraction (40 μg) to another batch of oocytes culture significantly increase the release of progesterone while the addition of 200 K supernatant fraction (40 μg) from control oocytes had no such effect. Protein was measured by following Lowry *et al.*[16] taking bovine serum albumin as the standard. Treatment of 200 K supernatant fraction from T_3-treated oocytes with trypsin destroyed its stimulatory effect on progesterone release (Table 2). Data therefore indicate that stimulation of progesterone release from the oocytes by T_3 is not its direct effect but mediated via a factor induced by T_3 which is proteinaceous in nature.

In conclusion it may be remarked that thyroid hormone receptor in perch oocyte nuclei has a physiological relevance as thyroid hormone could significantly stimulate progesterone release from the oocytes. But this action of thyroid hormone on perch oocyte is not direct, it induces the generation of a proteinaceous factor(s) which in turn stimulates progesterone release. To determine whether it is one or more than one type of proteins and to explain the detailed mechanism involved in this process require further investigations.

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Correction

Estimation of excited state dipole moment of substituted coumarins

R. Giri and M. M. Bajaj

(*Curr. Sci.*, 1992, **62**, 522–525)

M. M. Bajaj writes:

As I was not directly or indirectly connected with the conduct of the work reported in the paper, I hereby *withdraw my name from the coauthorship of the paper.* Much of the work reported in the paper was done in the department of M. K. Machwe, S. S. Rathi and V. V. S. Murti. I am also sorry for the inconvenience caused to the Editor of the journal.