phils is 3 to 6 times that of an equivalent area of extracellular space. The level of radioactivity in the extracellular space is highest 1 min after estrogen injection and falls off fairly rapidly thereafter.

Discussion. The dynamics of the in vivo binding and release of tritiated estradiol by the rat uterus were studied by dry radioautography in different cell types. High levels of radioactivity were found in the nuclei of epithelial, glandular, stromal and muscular cells and in the eosinophils shortly after the i.v. injection of the labelled estradiol. Peak levels of estradiol in the nuclei of luminal epithelial, glandular, stromal and muscular cells were reached between 5 and 20 min after the hormone injection.

At variance with present results, it was previously reported that the nuclear radioactivity in the immature rat uterus was apparent 15 min after estrogen injection, and that the maximum nuclear concentration was found at the end of 1 h[12]. However, in these studies, the hormone was injected s. c. and probably the absorption was slow and irregular. It is generally assumed that the binding of a hormone to its specific receptor must precede all the effects mediated by this association. In this context, the binding of estrogens by the uterine eosinophils at very early times is in accordance with the hypothesis that some of the early parameters of estrogen stimulation are mediated by the eosinophil receptor system[3-5,13-15]. The eosinophils were believed to be attracted directly by estrogens by a double-receptor mechanism[5,15], and once they have migrated to the uterus, they would mediate some estrogenic effects, such as water imbibition, increase in vascular permeability, histamine release and estrogen priming effects[3-5,13-15].

The early binding of estrogens to the cytosol-nuclear receptor system may explain some of the other estrogenic effects, such as the early synthesis of mRNA and the early increase in the incorporation of amino acids into proteins by estrogens[16,17], which, in turn, would precede other genomic effects of estrogens. A few other estrogenic effects, such as the early increase in cyclic AMP, cannot be attributed at this moment to any known receptor system of estrogens, and its mechanism of production and its function in uterine physiology remains to be elucidated.

12 W. E. Stumpf, C. Baerwaldt and M. Sar, in: Basic Action of Sex Steroids on Target Organs; 3rd International Seminar on Reproductive Physiology and Sexual Endocrinology p. 3. (Ed. P. O. Hubinont, F. Leroy and P. Galand), Karger, Basel 1971.
13 A. Tchernitchin, J. Rooryck, X. Tchernitchin, J. Vandenhende and P. Galand, Molec. cell. Endocr. *2*, 331 (1975).
14 A. Tchernitchin, X. Tchernitchin and P. Galand, Experientia *31*, 993 (1975).
15 X. Tchernitchin, A. Tchernitchin and P. Galand, Differentiation *5*, 151 (1977).
16 J. Gorski and N. J. Nelson, Archs Biochem. Biophys. *110*, 284 (1965).
17 A. R. Means and T. H. Hamilton, Biochim. biophys. Acta *129*, 432 (1966).

Bovine TSH-stimulation of fish thyroid peroxidase activity and role of thyroxine thereon

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Summary. bTSH augmented the fish thyroid peroxidase activity in a dose-response manner. Thyroxine could not modulate the effect of exogenous bTSH, but it decreased the peroxidase activity in a control system when administered alone. The data therefore suggest similar negative feedback control system for TSH-regulation as operative in the case of mammals.

Thyrotrophin (TSH) is known to stimulate the thyroidal activity in a number of ways, such as activation of adenyl cyclase[3,4], increased cyclic AMP-production[5,6], hydrolysis of thyroglobulin[7], synthesis of specific thyroid proteins[8] and total RNA-synthesis[9-12]. It is evident from these reports that TSH-action is directed towards the formation of specific thyroidal enzymes involved in the oxidation and incorporation of iodide into the tyrosine moiety, which eventually leads to the formation of thyroid hormones. It has also been reported that, in the case of the pituitary thyroid axis, excess thyroid hormone exerts a direct inhibitory effect on TSH-secretion, interacting with the stimulatory effects of thyrotrophin releasing hormone (TRH)[13,14]. On the basis of above reports from mammalian sources, we attempted to demonstrate the role of bovine TSH on teleostean fish thyroid peroxidase activity and existence of a negative feed-back control system for TSH-action. We have reported earlier that the teleost pharyngeal thyroid microsomal preparation contain physiologically important peroxidase, since the same fraction could catalyse iodonation and thyroxine formation[15-17].

Materials and methods. *Ophicephalus gachua*, a commonly available teleost fish in India, was purchased from the local market and kept in the aquarium for at least 1 week before use. For each experiment 5–6 fishes were chosen, injected i.p. with bovine TSH or Thyroxine (T_4) or TSH plus T_4 or distilled water (which serves as control), and kept separately in different aquaria for 6 h, and then sacrificed. Pharyngeal portion was carefully dissected out and homogenized in a Potter-Elvehjem homogenizer in 0.05 M Na-phosphate buffer, pH 5.0, then subjected to ultracentrifugation for collection of microsomal fraction which is designated as enzyme preparation. The details of the procedure has been given in some earlier reports[16,18]. Peroxidase activity of the enzyme preparation was measured by following the increase of OD at 470 nm in a spectrophotometer (spectronic 20, Bausch & Lomb) using 1 cm light path with guaiacol as hydrogen donor. The reaction mixture contained 150 µmoles of Na-phosphate buffer, pH 5.0, 1 µmole of guaiacol, 15 µmoles of H_2O_2 and enzyme preparation in a suitable volume of water to make final volume 3.0 ml. Enzyme protein was measured according to the method of Lowry et al.[19], using bovine serum albumin as the standard.

Results and discussion. To observe the TSH-augmentation of fish thyroid peroxidase activity, a time course response was studied by injecting 0.5 unit of TSH per 100 g b.wt and fishes were sacrificed in regular 2-h-intervals from 2 to 10 h. From each group, enzyme was then prepared

and assayed for peroxidase activity. It could be seen from figure 1 that TSH increases the peroxidase activity up to 6 h almost linearly, and then the stimulatory effect of TSH is gradually withdrawn up to 8 h and a sharp fall was noted at 10 h. In mammals, it has recently been shown that 4-h-treatment of TSH could stimulate the increase turnover as well as synthesis of thyroidal poly-(A)-RNA and nonpoly-(A)-RNA[20], suggesting a rapid increase in protein synthesis due to TSH-induction. However, in the case of fish, there is no information on the point, but our data show that there was a marked augmentation of thyroid peroxidase activity which might be due to the stimulation of thyroidal protein synthesis at early hours.

Thyroxine role on TSH-augmented fish thyroid peroxidase activity

System	Δ OD/min mg protein (Mean ± SE)	Stimulation (%)	Inhibition (%)
Control	0.19 ± 0.007***	—	—
TSH*-treated	0.32 ± 0.003***	68.4	—
TSH*-T_4**-treated	0.32 ± 0.094	68.4	—
T_4-treated**	0.07 ± 0.015***	—	63.1

* 0.5 unit/100 g b. wt; ** 50 µg/100 g b. wt; ***Difference between control and TSH-treated and between control and T_4-treated is significant at $p < 0.001$.

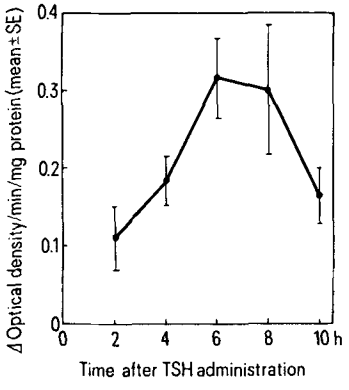

Fig. 1. Time course study of TSH-stimulation of fish thyroid peroxidase activity.

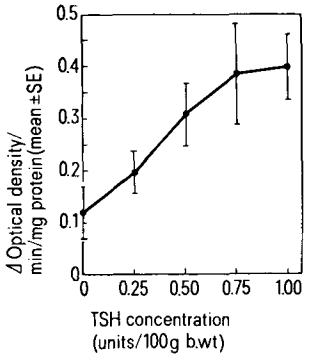

Fig. 2. Effect of varied doses of TSH on fish thyroid peroxidase activity.

Figure 2 denotes the effect of varied TSH-dose-responses in thyroid peroxidase activity at 6-h-level. TSH at the dose from 0.25 to 0.75 unit/100 g b.wt of fishes results a proportional increase of peroxidase activity and then the activity levels of at 1.0 unit/100 g b.wt. It has recently been reported that, in the case of mouse thyroid, higher concentration of TSH does not produce any increase in its response[21]. In our case also, increase of TSH-concentration from 0.75 to 1.0 unit/100 g b.wt had practically no effect on the peroxidase activity.

It is known in mammalian system that the prior injection of thyroid hormone abolished the stimulation of TSH-secretion braught about by TRH-injection[22,23], therefore demonstrating the existance of a negative feedback control on TSH-release. Whether the similar negative feed back control system for TSH-regulation is also operative in the case of fish is not known. We therefore attempted to investigate this in our experimental model. Seperate groups of fishes received the i.p. injection of either TSH alone or TSH plus T_4, or only T_4, or distilled water (which served as a control) and sacrificed after 6 h. Results of this experiment can be seen in the table. Augmentation of fish thyroid peroxidase activity was effected both by TSH and TSH plus T_4, which indicates that exogenously supplied TSH-action remains unaffected by subsequent high dose of T_4. But when only T_4 was administered in the same concentration, it depressed the peroxidase activity markedly in comparison with the control (table). This might be due to the thyroxine-mediated inhibitory effect on endogenous TSH-secretion.

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2 Reprint requests to: S. B.

3 I. Pastan and R. Katzen, Biochem. biophys. Res. Commun. *29*, 792 (1967).
4 G. Burke, Endocrinology *86*, 353 (1970).
5 T. Tonoue, W. Tong and V. Stolc, Endocrinology *86*, 271 (1970).
6 J. A. William, Endocrinology *91*, 1411 (1972).
7 P. L. Poffenbarger, R. C. Powell and W. P. Deiss, J. clin. Invest. *42*, 239 (1963).
8 J. Delarco and G. Guroff, Biochem biophys. Res. Commun. *49*, 1233 (1972).
9 R. Ekholm and V. Pantic, Nature, Lond. *199*, 1203 (1963).
10 R. H. Lindsay and P. P. Cohen, Endocrinology *76*, 737 (1965).
11 R. E. Lecocq and J. E. Dumont, Biochem biophys. Acta *129*, 421 (1966).
12 R. R. Kerkof and J. R. Tata, Biochim. J. *112*, 729 (1969).
13 S. Reichlin, Archs intern. Med. *135*, 1350 (1975).
14 G. F. Grant and W. Vale, Curr. Topics exp. Endocr. *2*, 38 (1974).
15 S. N. De and S. Bhattacharya, Ind. J. exp. Biol. *14*, 561 (1976).
16 S. Bhattacharya, P. Dasgupta and D. Kumar, Ind. J. exp. Biol. *14*, 227 (1976).
17 S. Bhattacharya, R. H. Das and A. G. Datta, Gen. comp. Endocr. *30*, 128 (1976).
18 D. Kumar, P. Dasgupta and S. Bhattacharya, Experientia *29*, 1076 (1973).
19 O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Rendall, J. biol. Chem. *193*, 265 (1951).
20 J. Y.-L. Yu, S. Bhattacharya and A. Gorbman, Life Sci. *19*, 1089 (1976).
21 M. Gafni and J. Gross, Endocrinology *97*, 1486 (1975).
22 J. B. Martin and S. Reichlin, Endocrinology *90*, 1079 (1972).
23 P. J. Snyder and R. D. Utiger, J. clin. Invest. *51*, 2077 (1972).