Therefore, the ACTH-induced increase in the half-life of adrenocortical mitochondrial proteins could be explained by assuming that this hormone elicits a higher rate of neutralization of the label. However, this possibility can be disregarded, since this effect of ACTH is not inhibited by injecting the animals with daily doses of L-leucine, which presumably would decrease the tracer concentration in the intracellular aminoacidic pool.

On this ground, the CAP-induced inhibition of the ACTH-enhanced half-life of adrenocortical mitochondrial proteins indicates that this effect of the hormone requires the continuous synthesis (possibly stimulated by ACTH) of protein(s) translated by the CAP-sensitive mitochondrial enzymatic machinery. We advance the hypothesis that such protein(s) might be involved in the attachment to the mitochondrial membranes (and, therefore, in the stabilization) of the enzymatic proteins synthesized at the microsomal level.

Stereological evidence indicates that a chronic treatment with ACTH induces, in the rat adrenal cortex, a significant increase in the surface of the mitochondrial cristae[10], which can be interpreted as a morphological counterpart of the ACTH-enhanced growth and steroidogenic capacity of adrenocortical cells; in fact, numerous enzymes of the steroid-synthesis are inserted on the mitochondrial cristae[11]. The present data appear to indicate that the mechanism underlying this trophic effect of ACTH involves not only the stimulation of nuclear and mitochondrial DNA-dependent protein synthesis[9], but also the stabilization of the mitochondrial enzymatic and structural proteins.

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Dynamics of estrogen binding by uterine cells in vivo[1]

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Summary. The dynamics of the in vivo binding and release of tritiated estradiol in different uterine cell types are described. The very early binding of estrogens by the cytosol-nuclear and the eosinophil receptor systems is in accordance with the hypothesis that some estrogenic effects are mediated by these receptor systems.

The current hypothesis on the mechanisms of estrogen action assumes the binding of the steroid to the specific receptors on target cells[2,3]. This binding triggers a series of events, which constitute the response to estrogens[2,4,5]. According to this hypothesis, the binding of estrogens to their receptors must occur before any change in any parameter of estrogen stimulation. The kinetics of the events that occur in the uterus after estrogen stimulation have been studied in detail[5]. Early increases in uterine cyclic AMP[6] and in the number of uterine eosinophils[5] have been found in vivo min after estrogen administration. There is no information, however, on the kinetics of in vivo estrogen binding to the receptors in different uterine cells. The present report describes the dynamics of in vivo estrogen binding and release by various rat uterine cell types.

Material and methods. Female Sprague-Dawley rats, weighing 200 g, in estrus and on the 1st day of diestrus, were used. Tritiated estradiol-17β (500 μCi, corresponding to 1.4 μg) was injected into the jugular vein and the animals were killed at various times after the estrogen injection. The uteri were excised and processed by a dry radioautographic technique for soluble compounds designed by Gersh[7–10] and modified by Stumpf[11]. The radioautograms were developed after 3 months of exposure and stained with hematoxylin-eosin or methyl green-pyronine.

For each experimental condition and for each cell type, radioautographic granules over 40 cells (and/or nuclei) were counted. The radioactivity of the 'extracellular space' was estimated by counting 40 areas of a size comparable to an average eosinophil, marked by a circle in the ocular piece of the microscope in areas chosen at random between cells located in the deep stroma.

Results. The figure shows the dynamics of estrogen binding and release by the different uterine cell types. Glandular, stromal and muscular cells appear to follow a common pattern of uptake of radioactive estradiol. Very little concentration of radioactivity over the nuclei of the connective tissue cells is observed within 1 min after the injection. However, in the vicinity of the blood vessels, there is a clear tendency for localization of radioactivity in the nuclei of stromal and glandular epithelial cells. 3 min after the injection of the labelled steroid, there is already a clear localization on the nuclei of glandular, stromal and muscular cells. The amount of radioactivity increases with time, and reaches its maxi-

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mum between 5 and 20 min after the injection. At 20 min after the injection, the radioactivity begins to decrease in the nuclei of the glandular, stromal and muscular cells. However, 2 h after the injection, considerable radioactivity still remains.

The dynamics of binding and release of tritiated estradiol in the nuclei of luminal epithelial cells during estrus is similar to that described in the glandular, stromal and muscular cells. In animals in the 1st day of diestrus, however, the luminal epithelial cell nuclei show very little uptake of labeled estradiol. The radioactivity that does appear reaches its maximum level between 5 and 20 min after the injection and falls off fairly rapidly thereafter.

The uptake of tritiated estradiol by uterine eosinophils is already significant 1 min after estrogen injection. The maximum level of radioactivity in eosinophils occurs between 3 and 5 min after the injection of the isotope. At that time, the radioactivity in eosinophils is approximately 3 to 4 times that of an equivalent area of extracellular space. After 5 min, the radioactivity in eosinophils decreases continuously, but its concentration in eosino-

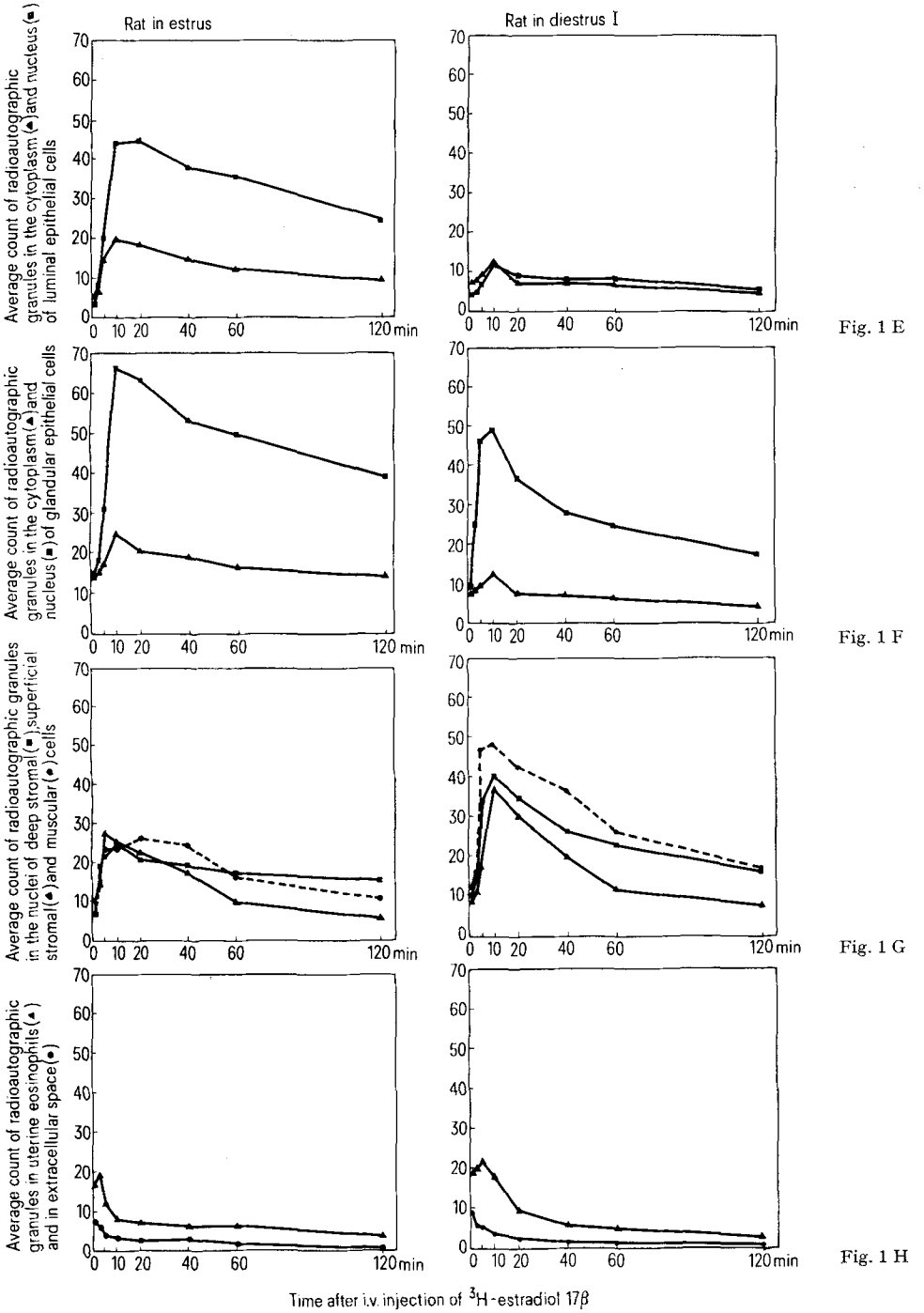

Fig. 1 A
Fig. 1 B
Fig. 1 C
Fig. 1 D
Fig. 1 E
Fig. 1 F
Fig. 1 G
Fig. 1 H

Dynamics of in vivo ^{3}H-estradiol-17β binding and release in different uterine cell types. Results are expressed as average counts of radioautographic granules in various uterine cell types in rats in estrus and in the 1st day of diestrus.

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.

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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