

The immunoreactive ACTH intracellular contents did not change significantly after the 72 h incubation time. High immunoreactive ACTH concentrations were measured in all the incubation media samples changed at the various periods (Figure 2, right side). However, the ACTH-like protein immunoreactivity of the contents of the samples decreased successively with the increase of the incubation time and a significant difference was found between the 24 and the 72 h samples.

Moreover, the total ACTH-like immunoreactivity measured in the medium was nearly three times that found in the placental fragments before initiating the culture ( $P < 0.001$ ).

**Discussion.** The results demonstrate the production, by human placental cells and amniotic membranes, of an ACTH-like peptide or protein which cross-reacts in a homologous human-anti-human ACTH radioimmunoassay. These observations confirm those of the previous studies on the presence of an ACTH-like substance in pregnancy plasma and in human placental extracts<sup>6,7</sup>. Moreover, they demonstrate that this substance is synthesized in the placenta itself, rather than coming from maternal blood.

The different results reported here and in the previous studies, and based both on the immunological activity of this ACTH-like substance, and demonstrating its placental origin, justify its proposed name: human chorionic corticotrophin (HCC). The use of a partial cross-reaction in the radioimmunological system does not permit an exact evaluation of the concentration of the substance. However, the observation that the total immunoreactive ACTH secreted in the medium corresponds to three times the intracellular content before culture strongly suggests that HCC synthesis is, in the experimental conditions used, very active. The demonstration of amniotic membrane content and secretion capacity of both HCS and HCC suggests a model for the study of the role of these cells in the protein composition of amniotic fluid.

In conclusion, the present data demonstrate the capacity of the placental and amniotic cells to synthesize and secrete

in vitro, besides HCS, human chorionic corticotrophin. The importance and the role of the latter in human reproduction is open to much speculation and must be investigated by further research<sup>18</sup>.

**Résumé.** La synthèse de la somatomammotrophine (HCS) et d'un facteur présentant une réaction immunologique croisée avec l'ACTH a été démontrée dans des cultures de fragments de placenta et de membrane amniotique. Ce facteur est comparable à la corticotrophine chorionique humaine précédemment décrite dans le plasma de femme enceinte et les extraits placentaires.

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## Effects of Thyroidectomy on Ovarian Compensatory Hypertrophy in Rats

Several investigators have studied the effect of thyroidectomy (or propylthiouracil-induced hypothyroidism) on the hypertrophy of various organs following unilateral removal of the partner organ. ZECKWER<sup>1</sup> showed that thyroidectomy reduces the weight of the remaining adrenal of unilaterally adrenalectomized rats below that of intact controls but does not affect the compensatory response of the remaining kidney in the

unilaterally nephrectomized rat (ZECKWER<sup>2</sup>). Also, PROULX and GORSKI<sup>3</sup> demonstrated that in unilaterally adrenalectomized rats rendered anovulatory by administration of androgens, the weight of the remaining adrenal is significantly reduced by administration of propylthiouracil. CONTOPOULOS and KONEFF<sup>4</sup> revealed that removal of the thyroid in intact female rats caused a reduction in ovarian weight and recently SAIDUDDIN<sup>5</sup>,

Effect of thyroidectomy and/or hemicastration on body and ovarian weights and corpora lutea production

Group	Condition	No.	Body weights (g) (Mean $\pm$ S.E.)	Left ovarian weights (mg) (Mean $\pm$ S.E.)	Relative ovarian weight <sup>a</sup>	Number of corpora lutea (Mean $\pm$ S.E.)
I	Intact	46	224.2 $\pm$ 2.1	39.8 $\pm$ 1.1	0.178 $\pm$ 0.0045	13.1 $\pm$ 0.65
II	Hemicastrated	49	218.5 $\pm$ 2.6 <sup>a</sup>	58.9 $\pm$ 1.6 <sup>c</sup>	0.269 $\pm$ 0.0059 <sup>c</sup>	18.5 $\pm$ 0.76 <sup>c</sup>
III	Hemicastrated + thyroidectomized	46	202.3 $\pm$ 3.2 <sup>b</sup>	49.9 $\pm$ 1.4 <sup>b</sup>	0.247 $\pm$ 0.0067 <sup>b</sup>	15.6 $\pm$ 0.93 <sup>b</sup>

<sup>a</sup> Significantly different ( $p < 0.05$ ) from group III. <sup>b</sup> Significantly different ( $p < 0.05$ ) from groups I and II. <sup>c</sup> Significantly different ( $p < 0.05$ ) from groups I and III. <sup>d</sup> Left mg ovarian weight/g body weight.

using hemicastrate female rats showed that hypothyroidism reduces weight but does not completely block the hypertrophy of the remaining ovary. The purpose of the present study was an effort to explain the effect of thyroidectomy upon weight of the remaining ovary in the hemicastrate rat through a histological examination of the ovary.

Adult female rats (160–180 g) were divided into 3 groups. The first group was left intact to serve as controls, while the second group was unilaterally ovariectomized. In the last group, both the right ovary and the thyroid were removed. Animals were killed 14 days after operation. At autopsy, the left ovary was removed, weighed and fixed in formalin for histological evaluation.

Hemicastration was followed by a significant increase (48%) in weight of the remaining ovaries over those of the intact controls (Table). Removal of the thyroid partially blocked the compensatory hypertrophy; the mean ovarian weight of the hemicastrate-thyroidectomized rats (49.9 mg) was significantly greater than that of intact controls (39.9 mg) yet was significantly smaller than those of the himicastrate group (58.9 mg).

The lower body weight in the thyroidectomized group may reflect a reduced food intake and WILLIAMS<sup>6</sup> has stressed this effect on compensatory hyperplasia. However, when the ovarian weights in the present study were expressed as percentages of total body weight, the resulting proportionate weights of the hemicastrate-thyroidectomized group were significantly different from those of both the intact and hemicastrate groups. Preliminary results from our laboratory suggest that administration of thyroxine (10 µg/day) to unilaterally ovariectomized-thyroidectomized rats has no effect on ovarian weight (50.5 mg).

PETERSON, EDGREN and JONES<sup>7</sup> demonstrated that ovaries of hemicastrate rats have an increase number of corpora lutea which may account for the increased weight of the remaining ovary. In the present study, removal of the thyroid significantly decreased the number of corpora lutea in the ovary of the hemicastrate. This reduction of corpora lutea partially explains the reduction in ovarian weight and may be a reflection of reduced gonadotropin release from the pituitary.

**Résumé.** Le nombre de corps jaunes dans l'ovaire de rates intactes, hémicastrées, hémicastrées et thyroïprivées fut déterminé. Les corps jaunes de l'animal thyroïprivé et hémicastré furent sensiblement plus petits que ceux de l'animal hémicastré. Cette diminution des corps jaunes se traduit par une diminution concomitante du poids ovarien et pourrait expliquer le blocage partiel de l'hypertrophie compensatrice du reste de l'ovaire.

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<sup>1</sup> I. T. ZECKWER, *Am. J. Physiol.* 123, 266 (1938).

<sup>2</sup> I. T. ZECKWER, *Am. J. Physiol.* 145, 681 (1946).

<sup>3</sup> R. P. PROULX and R. A. GORSKI, *Endocrinology* 77, 406 (1965).

<sup>4</sup> A. N. CONTOPOULOS and A. A. KONEFF, *Acta endocr., Copenh.* 42, 275 (1963).

<sup>5</sup> S. SAIDUDDIN, *J. Endocr.* 54, 371 (1972).

<sup>6</sup> G. E. G. WILLIAMS, *Nature, Lond.* 196, 1221 (1962).

<sup>7</sup> D. PETERSON, R. A. EDGREN and R. C. JONES, *J. Endocr.* 29, 255 (1964).

### Comparative Responsiveness of Euthyroid and Hypothyroid Rat Pituitary Tissue to Thyrotropin Releasing Hormone in vitro

The hypothalamic thyrotropin releasing hormone (TRH) has been shown to elicit a prompt release of thyrotropin (TSH) from pituitary glands of several species of euthyroid animals in vitro<sup>1-5</sup>. The present study compares the release of TSH from euthyroid and hypothyroid rat pituitary tissue with varying doses of synthetic TRH in vitro.

**Materials and methods.** Euthyroid and thyroidectomized female Sprague-Dawley rats weighing 150–200 g were obtained from Charles-River. The thyroidectomized rats were sacrificed 21 days after thyroidectomy; in all cases the rats were sacrificed by decapitation and the pituitary glands were immediately removed and placed in TC 199 medium (Difco). Each pituitary gland was then hemisected; one half was placed in a 'control' test tube containing 1.0 ml TC 199 medium while the corresponding half was placed in an 'experimental' test tube also containing 1.0 ml of TC 199. Preincubation proceeded for 30 min at 37°C with the medium renewed 1 time. At the preincubation period, each hemi-pituitary was placed in 1.0 ml of freshly oxygenated (95% O<sub>2</sub> 5% CO<sub>2</sub>) and pH adjusted (7.4) TC 199 medium containing either no additions (control hemi-pituitary) or 0.1, 1.0, 10.0, 100.0 or 1000.0 ng/ml synthetic TRH (Calbiochem) (experimental hemipituitary). In all cases a corresponding hemipituitary served as the control for the experimental hemipituitary. 4 rats were used for each dose of TRH. Incubation was then allowed to proceed at 37°C with gentle agitation every 5 min. 10 ml aliquots of the in-

cubation medium were obtained at 15, 30 and 60 min and immediately placed in medium for radioimmunoassay of rat TSH (rat TSH kit was obtained through the courtesy of Dr. A. PARLOW and NIAMD, Bethesda, Maryland). After each experiment the wet weights of the hemipituitaries were obtained by weighing on a Mettler balance to the nearest 0.01 mg. Serum thyroxine by competitive protein binding was undetectable in thyroidectomized animals.

**Results.** With increasing doses of TRH there is an increase in release of TSH with the maximum effect seen at 10–100 ng/ml TRH with both euthyroid and hypothyroid pituitaries. There was no significant difference between the responsiveness of euthyroid vs. hypothyroid pituitary tissue when exposed to 1.0, 10.0, 100.0, or 1000.0 ng/ml TRH at 15, 30, or 60 min. However, at 30 min and 60 min the response of the euthyroid pituitary tissue to 0.1 ng/ml TRH was significantly greater than

<sup>1</sup> R. GUILLEMIN, E. YAMAZAKI, D. GARD, M. JUTISZ and E. SAKIZ, *Endocrinology* 73, 564 (1963).

<sup>2</sup> A. V. SCHALLY and J. W. REDDING, *Proc. Soc. exp. Biol. Med.* 126, 320 (1967).

<sup>3</sup> J. F. WILBUR and R. D. UTIGER, *Proc. Soc. exp. Biol. Med.* 127, 488 (1968).

<sup>4</sup> F. S. LABELLA and S. R. VIVIAN, *Endocrinology* 88, 787 (1971).

<sup>5</sup> P. B. MAY and R. K. DONABEDIAN, *J. clin. Endocr. Metab.* 36, 605 (1973).