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Mechanism of Pituitary Gonadotropic Antagonism.

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The capacity of certain pituitary extracts to act as gonadotropic antagonists upon intraperitoneal administration has been the object of several investigations.[1] We have recently shown, in collaboration with Dr. H. M. Evans and Dr. M. E. Simpson, that the antagonistic effect is due to the presence of the interstitial cell stimulating or luteinizing factor (ICSH, LH) in the pituitary preparation.[2, 3] We, therefore, assumed that the antagonistic factor is identical with the ICSH (LH). Fevold and Fiske have come to similar conclusions.[4] The mechanism and physiological significance of the gonadotropic antagonism is, however, still a matter of debate.

Recent investigations carried out in this laboratory indicate that the pituitary ICSH (LH) may be identical with the thyrotropic factor of the anterior pituitary since attempts to separate the two physiological effects by chemical means have so far been unsuccessful. This finding suggests that the secretion of the thyroid may be involved in the antagonistic action of the ICSH. It has been reported by various investigators[5, 6, 7] that the secretion of the thyroid inhibits the action of the follicle stimulating hormone (FSH) of the anterior pituitary. In order to correlate further the hypophyseal antagonistic effect with the secretion of the thyroid, we studied the influence of thyroxine on the ovarian response of certain gonadotropic principles under experimental conditions similar to those used in our investigations on the ICSH antagonism.

Experimental. Twenty-one to 23-day-old normal rats, weighing from 35 to 50 g were made hyperthyroid by the administration of varying amounts of thyroxine. As gonadotropic agents we used a

1 Van Dyke, H. B., *The Physiology and Pharmacology of the Pituitary Body*, University of Chicago Press, Chicago, Ill., Vol. 2, 1939.

2 Jensen, H., Simpson, M. E., Tolksdorf, S., and Evans, H. M., *Endocrin.*, 1939, **25**, 57.

3 Evans, H. M., Simpson, M. E., Tolksdorf, S., and Jensen, H., *Endocrin.*, 1939, **25**, 529.

4 Fevold, H. L., and Fiske, V. M., *Endocrin.*, 1939, **24**, 828.

5 Smith, P. E., and Engle, E. T., *Anat. Rec.*, 1930, **45**, 278.

6 Fluhmann, C. F., *Am. J. Physiol.*, 1934, **108**, 498.

7 Leonard, S. L., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 599.

purified pregnant mare serum preparation and a pituitary FSH. The ovarian response of the hyperthyroid animals to these gonadotropic factors was diminished in all instances as compared to the ovarian response of normal animals, 5 to 6 rats being used in each group.

The inhibitory action of thyroxine on the effect of a gonadotropic preparation was demonstrated (1) upon simultaneous injection of both factors for 3 days either separately or mixed *in vitro,* and (2) upon treatment with thyroxine for 3 days previous to the administration of FSH. Table I gives an example of a 3-day test where thyroxine and pregnant mare serum were mixed *in vitro* and injected intraperitoneally, resulting in a definite reduction of the ovarian weights. Table II illustrates the antagonistic effect of thyroxine against hypophyseal FSH produced by subsequent administration of thyroxine and FSH, both given subcutaneously. A total dose of 0.05 mg of thyroxine was sufficient to diminish follicular development and to inhibit formation of an estrous uterus. Similar effects were observed when the thyroxine treatment was extended over a period of 8 days previous to the injection of FSH. It may be mentioned here that the inhibitory effect of thyroxine was observed on both subcutaneous and intraperitoneal injections while pituitary antagonism exerts itself only upon intraperitoneal administration.

The findings recorded in this paper are in agreement with results of similar experiments reported by different investigators. Fluh-

TABLE I.

Ovarian Response to Intraperitoneal Injection of Thyroxine and Pregnant Mare Serum, Mixed *in Vitro.* Autopsy performed 72 hours after onset of injection.

Thyroxine total dose, mg	Thyroxine alone, intrap., mg	PMS alone, intrap., mg	Thyroxine + PMS, mg	Controls, mg
0.4	9.8 SF	130 LF, CL	59 LF, CL	12 SF
0.2	—	130 LF, CL	74 LF, CL	—

Abbreviations: PMS, pregnant mare serum preparation (total dose 1 mg); SF, small follicles; LF, large follicles; CL, corpora lutea.

TABLE II.

Ovarian Response to Successive Subcutaneous Injection of Thyroxine and FSH.* All autopsies performed on day 7.

Thyroxine total dose, mg	Thyroxine alone day 1-3, mg	FSH* alone day 4-6, mg	Thyroxine day 1-3 + FSH day 4-6, mg	Controls mg
0.2	17 SF	43 MF	24 SF	17 SF
0.05	19 SF	—	21 S, MF	—

* Total dose 0.2 mg = 8 RU. SF, small follicles; MF, medium sized follicles.

mann[6] has shown that thyroid feeding decreases the effectiveness of pituitary gonadotropic preparations in rats. On the other hand, thyroidectomy will increase the effectiveness of hypophyseal gonadotropic fractions, but will not augment the response of pregnant mare serum,* chorionic gonadotrophin† and gamone.‡ [6, 8, 9] Tyndale and Levin[10] have examined the ovarian response of FSH preparations from menopause urine in normal, hypophysectomized and thyroxine-treated hypophysectomized rats. They observed that the physiological response of their FSH preparation is more limited in normal than in hypophysectomized rats for subluteinizing doses and believe this to be due to an inhibitory effect of the thyroid on follicular development. They also reported that administration of thyroxine to hypophysectomized rats reduces the response to menopause FSH.

The observations recorded indicate that the thyroid secretion can act as a physiological antagonist to follicular development. FSH activity is inhibited by thyroxine in normal rats (Table II) and in hypophysectomized rats (Tyndale and Levine). FSH activity is enhanced in the absence of the thyroid secretion, *i. e.*, in hypophysectomized and in thyroidectomized rats. Gonadotropic pituitary preparations having an effect on the thyroid, will elicit a greater ovarian response in thyroidectomized animals.[6] Gonadotropic preparations having no appreciable effect on the thyroid such as pregnant mare serum, gamone and the pregnancy urine principle, give the same ovarian response after thyroidectomy.[6, 9] In agreement with this latter observation is the finding that chorionic gonadotrophin, gamone and prospermin§ also fail to act as antagonists when given intraperitoneally in combination with pregnant mare serum. Unfractionated anterior pituitary extracts and hypophyseal ICSH are potent antagonists under these conditions.[3]

If the conception is correct that the secretion of the thyroid is involved in the mechanism of antagonism the inhibitory effect of ICSH should be less pronounced in thyroidectomized animals. Preliminary experiments in thyroidectomized rats|| have shown that a

* Containing predominantly LH and some FSH.

† Containing LH only.

‡ A preparation from pooled samples of menopause urine containing LH and FSH.

8 Leonard, S. L., and Hansen, I. B.. *Anat. Rec.*, 1936, **64**, 203.

9 Leonard, S. L., and DeFrates, J., *Endocrin.*, in press.

10 Tyndale, H. H., and Levin, L., *Am. J. Physiol.*, 1937, **120**, 486.

§ A preparation from pooled samples of normal male urine, containing LH and traces of FSH.

|| We are greatly indebted to Dr. J. DeFrates of the Endocrine Laboratory for performing the thyroidectomy on immature rats for us.

combination of ICSH and FSH, given intraperitoneally, does not produce the same degree of antagonism in the operated animals as in the controls.

It should be pointed out that the antagonism of hypophyseal ICSH and thyroxine has so far been demonstrated in the immature rat only. We have injected, intraperitoneally, a combination of pregnant mare serum and ICSH into adult female rats with established cycles and were unable to produce any appreciable antagonism. The question whether gonadotropic antagonism can be observed in immature or mature male rats has not yet been studied.

Brief reference should also be made to the observations of Fevold, Hisaw and Greep[11] and Fevold and Fiske[4] who have studied the effect of estrogen treatment on the response to hypophyseal FSH. They find the FSH activity enhanced after short treatment with estrogen but inhibited after prolonged treatment. They explain their findings by the outpouring of varying amounts of LH from the pituitary under the stimulus of estrogen administration. Their observation that normal ovaries implanted into estrogen-treated animals fail to respond to FSH administration may be explained in the light of our assumption that the luteinizing factor stimulates the thyroid, *i. e.*, estrogen-treated animals may be considered to be in a hyperthyroid state.

It has been reported[3] that purified FSH preparations also show antagonism when given intraperitoneally in combination with pregnant mare serum. This phenomenon is, however, observed only in normal rats while the antagonism of hypophyseal ICSH against pregnant mare serum can be obtained in hypophysectomized as well as in normal rats. It is probable that the antagonistic effect observed with FSH is due to the formation and liberation of estrogen which in turn acts on the pituitary. In order to confirm this possibility, we have given α-estradiol¶ simultaneously with pregnant mare serum to normal rats in the 72-hour test and observed a significant reduction in the response of pregnant mare serum.

Conclusions. The observation that the secretion of the thyroid exerts an antagonistic effect on the ovarian response of certain gonadotropic principles agrees with our assumption of the identity of the thyrotropic factor with the pituitary interstitial cell stimulating hormone (ICSH or LH) and suggests a possible explanation for the mechanism of the antagonistic properties of the ICSH.

11 Fevold, H. L., Hisaw, F. L., and Greep, R., *Am. J. Physiol.*, 1935, **114**, 508.

¶ We are greatly indebted to Dr. E. Schwenk of the Schering Corporation for supplying us with this material.