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Regulation of Pituitary Gonadotrophic Secretion:* Inhibition by Estrogen or Inactivation by the Ovaries?

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The concept that the normal level of circulating estrogen inhibits pituitary gonadotrophic secretion has remained unchallenged long enough to have become the traditionally accepted explanation for control of the ovarian-pituitary axis. It is invoked to explain the rise of gonadotrophins following ovarian extirpation. It is also invoked to explain that the effect of administering estrogens is to suppress pituitary gonadotrophic secretion.

This view was questioned by Lauson, Heller and Sevringhaus[1] upon finding that α-estradiol administered in moderate doses did not prevent the postcastration rise of gonadotrophins in rats. Soon after it was found that doses of estrogen sufficient to alleviate menopausal symptoms and to restore the vaginal and endometrial histological appearance to normal did not lower the postmenopausal rise of urinary gonadotrophins in women.[2] The failure of physiological amounts of estrogen to suppress gonadotrophins was confirmed in castrated women.[3] Not only does substitution of female sex hormones in the female fail to suppress gonadotrophins but also substitution of physiological amounts of androgens in the male fails to suppress gonadotrophins. This has been noted in castrated male rats,[4] in the male climacteric,[5] and in eunuchoids.[6] These data do not substantiate the hypothesis that the normal level of circulating estrogen inhibits pituitary gonadotrophic secretion. (We recognize and have confirmed the fact that unphysiologically large doses of estrogens and androgens have a markedly inhibiting effect upon pituitary gonadotrophic potency).

Since the hypothesis of inhibition by estrogen proves to be inadequate in several situations, an alternative explanation is required. A tentatively satisfactory hypothesis is that in stimulating gonadal growth and secretion, gonadotrophins are altered by the ovary to such a degree that they become inactive. Seidlin's observations[7] and those of Heller, Heller and Sevringhaus[2] strongly suggest this possibility.

Data are presented in this communication indicating that the rise in gonadotrophins following castration is principally due to lack of gonadotrophin inactivation, and only in part due to lack of inhibition by estrogen.

The concept that a target-organ inactivates the specific hormone stimulating it is not entirely without precedent. An analogous situation was postulated for the thyroid-pituitary axis by Loesser[8] and substantiated by Seidlin.[7] It remained for the classical work of Rawson, Sterne and Aub[9] to con-

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TABLE I.

		Donor rats							Recipient rats*		
		Before autopsy			At autopsy						
		Vaginal smears		Ovarian wt					Uterine wt		
	No. of rats	Before operation	After operation	At operation mg	At autopsy mg	Ovarian appearance	Uterine appearance	Thymic wt, mg	With fluid, mg	Without fluid, mg	Ovarian wt, mg
Intact controls	22	cycling	cycling	—	49.9	normal	normal	154	162	89	10.3
Castrate "	29	"	atrophic	normal	—	—	all atrophic	290	92	92	92.4
Ovary-spleen† non-adhesion	25	"	"	49.0	149.5	homogenous corpora lutea	all atrophic	235	123	94	20.4
Ovary-spleen adhesion	24	"	cycling	50.5	32.9	normal	normal§	156	163	97	28.8
Ovary-spleen adhesion and non-adhesion 0.5-5.0 μg/day estradiol benzoate	26	"	estrus	62.3	14.9	atrophic	all estrus	132	53	45	11.2
Ovary-spleen adhesion and non-ahdesion 0.25-0.05 μg/day estradiol benzoate	13	"	cycling‡	48.0	21.5	normal	normal§	168	168	92	22.3
Control wts of assay animals									30	30	10.0

* Each recipient rat was injected with a suspension made from a single donor rat pituitary.
† Both ovaries were removed and autotransplanted to the spleen.
‡ Cycles were irregular.
§ Normal signifies that various stages of stimulation were encountered (from diestrus to estrus).

clusively demonstrate that thyroid and lymphoid tissues and not other tissues growing *in vitro,* were capable of inactivating thyrotrophic hormones.

Materials and Methods. Adult virgin female rats of the Sprague-Dawley strain, weighing 200-250 g, were divided into 6 donor groups as listed in Table I. Autotransplants were made by placing both ovaries in the spleen during a single operative procedure in the experimental groups.

Donor pituitaries were macerated, suspended in saline and injected twice daily subcutaneously into 22-24-day-old immature female rats of the same strain for 3 days. Autopsies of recipients were made 24 hours later. One donor gland was injected into one recipient rat.

Results are listed in Table I.

Discussion. Regulation of pituitary gonadotrophin secretion in *normal intact animals* (Fig. 1) could be accomplished by either (or both) of 2 mechanisms: (a) Inactivation of gonadotrophins incident to their usage by the ovary could lead to lowering of circulating gonadotrophins (at position 1 in the figures). (b) The level of circulating estrogen could determine the amount of pituitary gonadotrophic secretion; *i.e.,* as the estrogen level increases gonadotrophin secretion decreases and vice versa (at position 4 in the figures).

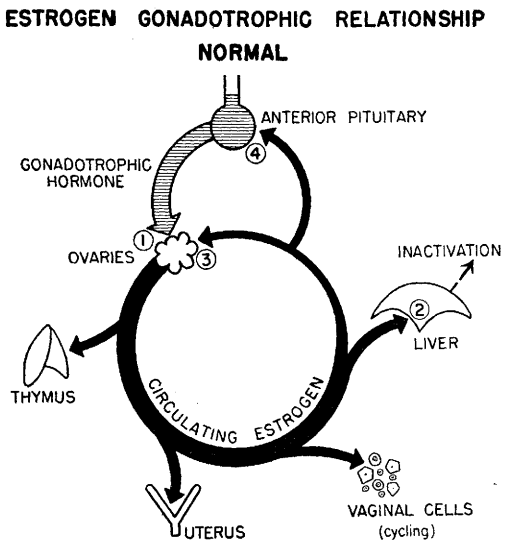

FIG. 1.

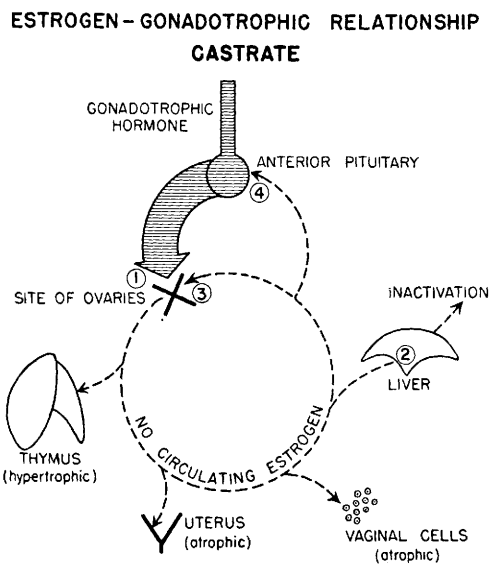

FIG. 2.

The rise in pituitary gonadotrophic potency from 10.3 mg (weight of ovaries of recipient assay animals) for the intact controls to 93.4 mg for the *castrate controls* (Fig. 2) can be explained by either or both of the 2 hypotheses: (a) Castration removes the organs (the ovaries) which ordinarily inactivate some of the circulating gonadotrophin during their normal activity. After castration, gonadotrophins are no longer removed from the circulation and therefore increase; consequently, pituitary gonadotrophic content increases. (b) Castration removes the organs (the ovaries) which ordinarily secrete enough estrogens to sufficiently inhibit pituitary gonadotrophic secretion and content so that levels are kept low. After castration, inhibition of pituitary gonadotrophic secretion and content are removed; consequently, pituitary gonadotrophic content increases.

In neither the intact animal nor the castrate animal can the 2 hypotheses be tested separately. However, administering estrogens in physiological replacement amounts will prevent other postcastration changes but will not prevent the postcastration rise in gonadotrophin content of the pituitary.[1] This observation is not consonant with the "inhibi-

tion by estrogen" hypothesis; but, other than being a permissive, it adds no support to the "inactivation by the ovaries" hypothesis.

In order to test the "inactivation by the ovaries" hypothesis, the ovary must have access to the circulating gonadotrophins, so that if inactivation of gonadotrophins occurs, this can be detected. At the same time estrogens must be prevented from reaching the pituitary, enabling its potency to rise in the event that this is the correct explanation. These circumstances are met by auto-transplantation of both ovaries to the spleen. Thus estrogens are elaborated directly into the portal circulation and are immediately inactivated by the liver. Thus no estrogens reach the anterior pituitary, and its estrogenic environment is identical to that of a castrate. On the other hand, the ovaries remain available to the gonadotrophins in the systemic circulation and could inactivate them. Under these circumstances the gonadotrophic content of the pituitary should distinguish between the 2 hypotheses. For example, if "inhibition by estrogens" alone is responsible for controlling pituitary gonadotrophic activity, then the pituitary gonadotrophins should be as high as that seen following castration, since in both instances the possibility of "inhibition by estrogen" has been removed. On the other hand, if "inactivation by the ovaries" alone is responsible for controlling pituitary gonadotrophic activity, then the pituitary gonadotrophins should be as low as in the intact controls. If both mechanisms are in operation, a value between these 2 extremes should result.

In the *experimental group* (auto-transplantation of both ovaries to the spleen) (Fig. 3) no estrogens reach the pituitary gland. The lack of circulating estrogen is indicated in Table I and Fig. 3 by the fact that thymic and uterine weights and vaginal cells are at castrate levels.

The crucial question is: Did the pituitary glands of the spleen-transplant rats contain amounts of gonadotrophins comparable to the intact control level of 10.3 mg or comparable to the castrate control level of 92.4 mg? The pituitary gonadotrophin assays of

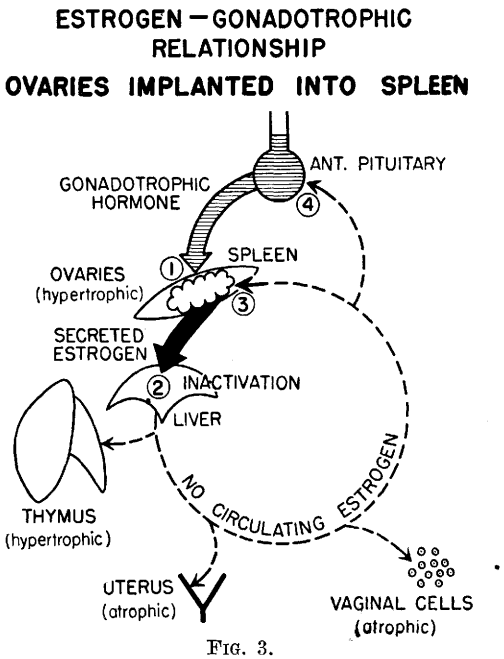

FIG. 3.

20.4 mg for the spleen-transplants clearly indicate that they are not comparable to the castrate controls (92.4 mg), but are comparable to the normal controls (10.3 mg). The gonadotrophin content is slightly but significantly elevated above normal.

From this it can be concluded that the primary regulation of pituitary gonadotrophin content is the removal of active gonadotrophins from the circulation by the ovaries. It must further be concluded that inhibition by estrogen plays a definite but minor role in this control.

Histological examination of the pituitaries of each of the 3 groups warrants the identical assumption insofar as the pituitaries of the experimental group (ovaries transplanted to spleen) exhibited cells reminiscent of "castration cells." These, however, are not as well developed as for comparable castrates.

Are the changes observed in the experimental group not simply incidental to manipulation attending the transfer of ovaries to the spleen? Some operated rats developed collateral circulation from the spleen (containing ovaries) to the systemic circulation by way of adhesions. These served as unwitting but admirable controls. The ovaries

in their new site were capable of elaborating estrogen in at least normal quantities as judged by the following findings: vaginal smears observed daily exhibited the same cycles as intact controls, thymus weights were identical to intact controls, and uterine development varied according to the stage of the cycle as in intact controls. It was somewhat puzzling therefore to find pituitary gonadotrophins slightly elevated above that of intact controls (28.8 mg). A possible explanation is that since the ovaries at first undergo partial atrophy following transplantation, and since they did not regain their preoperative size, they may have utilized less gonadotrophins. The eventual content of gonadotrophin in the pituitary seems to be a resultant of these 2 factors. This is further illustrated by administration of graduated amounts of α-estradiol to rats with ovaries transplanted to the spleen. Administration of large daily doses (5.0, 1.0 and 0.5 μg) for 34 days, from 6-18 days after operation to autopsy (Table I), caused total suppression of pituitary gonadotrophins. This was accompanied by complete ovarian atrophy for the 5.0 μg group. Had ovarian-utilization been the only mechanism in operation, the pituitary content would have been elevated to castrate levels. In contrast when smaller doses (0.25 and 0.05 μg) were administered, physiological levels of estrogen were approximated as judged by vaginal cells, uterine development and thymus weights. However, these dosages failed to permit ovarian growth (21.5 mg) to such an extent that they neither reached the spleen-transplant level of 149.5 mg nor their own operative control weight of 48 mg. Had inhibition by estrogen been the only mechanism in operation, the pituitary gonadotrophin content should have been as low as for intact controls (10.3 mg) instead of elevated to 22.3 mg. The elevation can be ascribed to failure of the smaller than normal ovaries to utilize the same amount of gonadotrophin as larger, normal ovaries would.

Summary. By autotransplantation of both ovaries into the spleen of mature female rats, the ovary continues to be bathed by pituitary gonadotrophins but the pituitary is no longer bathed by estrogens because in essence the liver has been inserted between the ovaries and the pituitary and has inactivated the estrogens.

The pituitaries of such rats were assayed for their gonadotrophic content. They more nearly resembled (20.4) that of intact controls (10.3), than that of castrated controls (92.4).

From this it was concluded that (1) ovaries normally inactivate gonadotrophins and that the rise of gonadotrophins seen following castration is due to failure of such inactivation to take place. (2) Large and unphysiological doses of estrogen are potent inhibitors of pituitary gonadotrophic potency. (3) Physiological amounts of estrogen in the circulation exert very little inhibitory action upon the pituitary.

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Regulation of Ovarian Growth: Inhibition by Estrogen or Stimulation by Gonadotrophins?

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In the preceding communication, it was demonstrated that the level of circulating gonadotrophic hormones fluctuates with the degree of ovarian activity. When ovaries are inactive or absent, they rise; when active, they fall. Perhaps ovarian growth and secretion are not solely regulated by the amounts of gonadotrophin present, but also by some other means. The results of estrogen administration to unilaterally castrated rats led