I. REGULATION OF PITUITARY GONADOTROPHIC SECRETION

II. REGULATION OF OVARIAN GROWTH

by

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REGULATION OF PITUITARY GONADOTROPHIC SECRETION: INHIBITION BY ESTROGEN OR INACTIVATION BY THE OVARIES?

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 in 1938 by Lausen, Heller and Severinghaus [1] upon finding that α-estradiol administered in moderate doses did not prevent the post-castration 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 post-menopausal 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 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 in-

adequate 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 Severinghaus [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 in 1934 [8] and substantiated by Seidlin in 1940.[7] It remained for the classical work of Rawson, Sterne and Aub [9] to conclusively demonstrate that thyroid and lymphoid tissues and not other tissues growing <u>in vitro</u>, were capable of inactivating thyrotrophic hormones.

<u>Materials and methods</u>. Adult virgin female rats of the Sprague-Dawley strain, weighing 200-250 grams, were divided into six 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 three 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 (Figure 1) could be accomplished by either (or both) of two 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).

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 (Figure 2) can be explained by either or both of the two 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 two hypotheses be tested separately. However, administering estrogens in physiological replacement amounts will prevent other post-

	No. of Rats	DONOR RATS							RECIPIENT RATS [1]		
		Before Autopsy				At Autopsy			Uterine Weight		Ovarian Weight
		Vaginal Smear		Ovarian Wt.							
		Before Operation	After Operation	At Operation	At Autopsy	Ovaries Appearance	Uterine Appearance	Thymic Weight	With Fluid	Without Fluid	
				mgm.	mgm.			mgm.	mgm.	mgm.	mgm.
Intact Controls	22	cycling	cycling	—	49.9	normal	normal	154	162	89	10.3
Castrate Controls	29	cycling	atrophic	normal	—	—	all atrophic	290	92	92	92.4
Ovary-Spleen [2] Non-adhesion	25	cycling	atrophic	49.0	149.5	homogenous corpora lutea	all atrophic	235	123	94	20.4
Ovary-Spleen Adhesion	24	cycling	cycling	50.5	33.9	normal	normal [4]	156	163	97	28.8
Ovary-Spleen Adhesion & Non-adhesion 0.5 -5.0 g/day Estradiol Benzoate	26	cycling	estrus	62.3	14.9	atrophic	all estrus	132	58	45	11.2
Ovary-Spleen Adhesion & Non-adhesion 0.25 - 0.05 g/day Estradiol Benzoate	13	cycling	cycling [3]	48.0	21.5	normal	normal [4]	166	168	92	22.3
Control Weights of Assay Animals									30	30	10.0

TABLE I

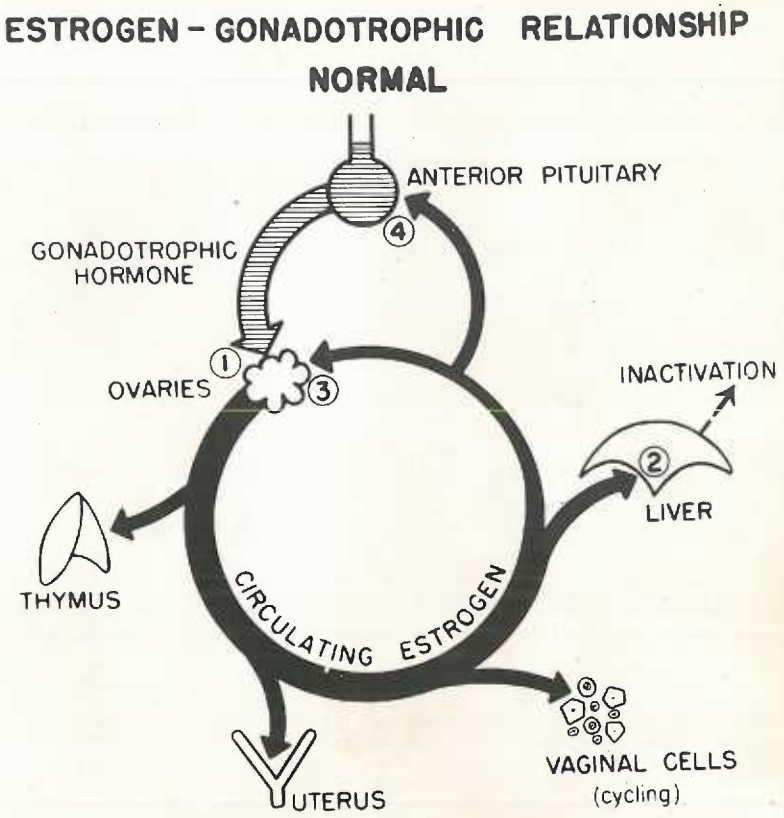
ESTROGEN – GONADOTROPHIC RELATIONSHIP
NORMAL
ANTERIOR PITUITARY
GONADOTROPHIC HORMONE
OVARIES
INACTIVATION
LIVER
THYMUS
CIRCULATING ESTROGEN
VAGINAL CELLS
(cycling)
UTERUS

Figure 1

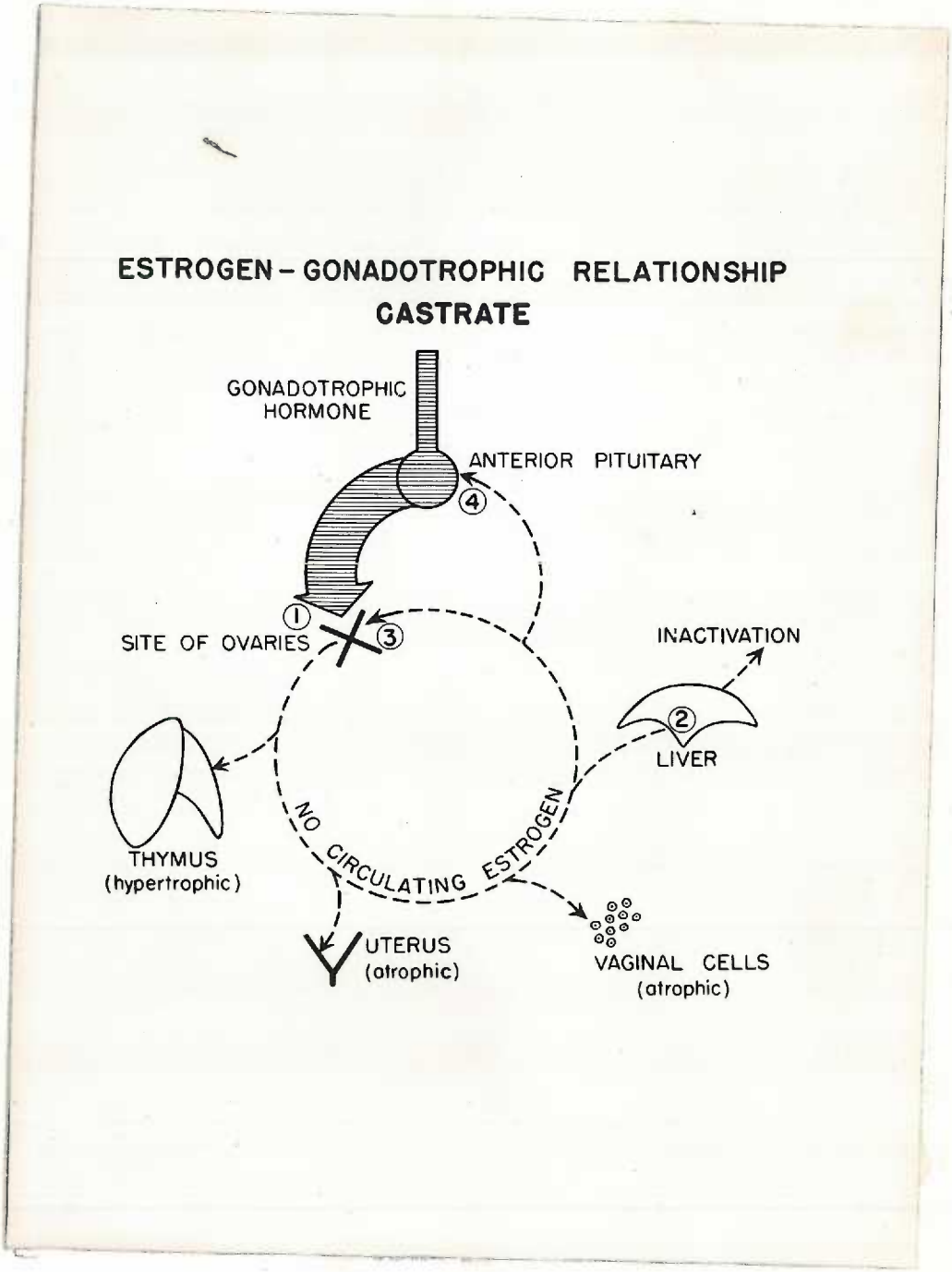
ESTROGEN – GONADOTROPHIC RELATIONSHIP
CASTRATE
GONADOTROPHIC HORMONE
ANTERIOR PITUITARY
4
1
SITE OF OVARIES
3
INACTIVATION
2
LIVER
THYMUS
(hypertrophic)
NO CIRCULATING ESTROGEN
UTERUS
(atrophic)
VAGINAL CELLS
(atrophic)

Figure 2

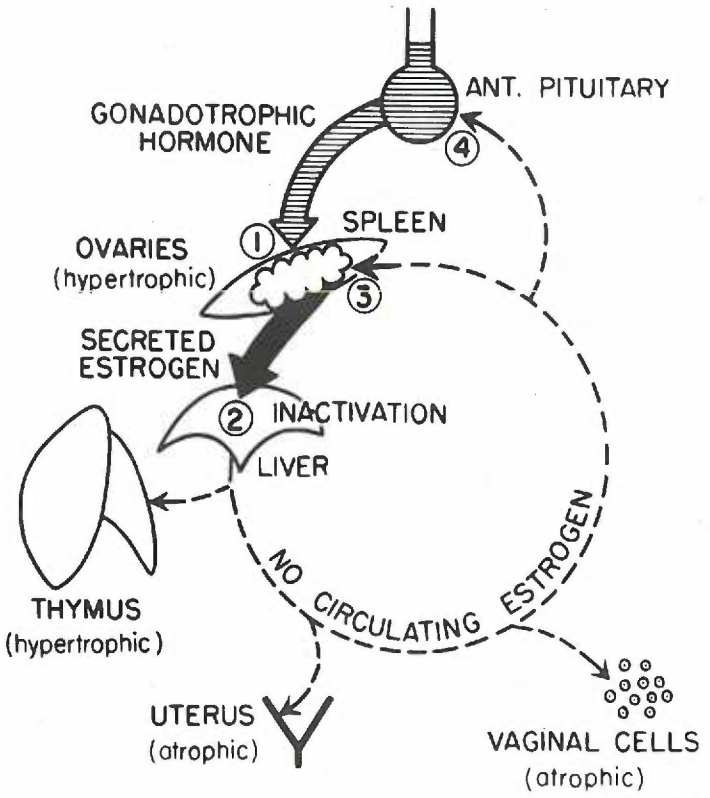
ESTROGEN — GONADOTROPHIC RELATIONSHIP
OVARIES IMPLANTED INTO SPLEEN
ANT. PITUITARY
GONADOTROPHIC HORMONE
4
SPLEEN
OVARIES (hypertrophic)
1
3
SECRETED ESTROGEN
2 INACTIVATION
LIVER
THYMUS (hypertrophic)
NO CIRCULATING ESTROGEN
UTERUS (atrophic)
VAGINAL CELLS (atrophic)

Figure 3

castration changes but will not prevent the post-castration rise in gonadotrophin content of the pituitary.[1] This observation is not consonant with the "inhibition 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 autotransplantation 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 two 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 two extremes

should result.

In the experimental group (autotransplantation of both ovaries to the spleen) (Figure 3) no estrogens reach the pituitary gland. The lack of circulating estrogen is indicated in Table I and Figure 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 93.4 mg.? The pituitary gonadotrophin assays of 20.4 mg. for the spleen-transplants clearly indicate that they are not comparable to the castrate controls (93.4 mg.), but are comparable to the normal controls (10.3 mg.). The gonadotrophin content is slightly but significantly elevated above normal.

From this is 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 three groups warrants the identical conclusion in that 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 two 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 (21.1 mg.) to such an extent that they neither reached the spleen-transplant level of 149.6 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.

REGULATION OF OVARIAN GROWTH: INHIBITION BY ESTROGEN OR STIMULATION BY GONADOTROPHINS?

In the preceding section, it was demonstrated that the level of circulating gonadotrophic hormone 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. In 1942 the results of estrogen administration to unilaterally castrated rats led Keller, Keller and Severinghaus [2] to suggest that the regulation of ovarian activity was the blood level of circulating estrogen. Thus a rise in blood level of estrogen could cause ovarian inhibition. Conversely a fall in blood level would remove inhibition and thereby stimulate the ovary.

Data are presented in this communication to indicate that estrogens secreted by the ovary act to inhibit growth of the ovary.

The concept that the circulating blood level of hormones secreted by a target-organ tends to regulate the production of hormone by that target organ is not without precedent. The analogous relationship between circulating thyroid-hormone and the production of thyroid-hormone was presented by Galli-Mainini.[10]

Control of ovarian growth and secretion in a normal intact animal may be by either (or both) of two mechanisms: (a) Stimulation of growth by pituitary gonadotrophin secretion (at position 1 in Figure 1) and (b) active suppression of growth by estrogenic inhibition (at position 3 in Figure 1).

To study this problem, a preparation must be devised which will separate these two possibilities. By autotransplanting the ovaries to

the spleen, the ovaries remain under the influence of pituitary gonadotrophin stimulation; however, the estrogen secreted by the ovaries in the spleen is carried by the portal vein to the liver and inactivated, thus removing the ovaries from the effects of circulating estrogen. This is confirmed by the castrate appearance of the thymus, the uterus and the vagina of the experimental animals.

Materials and methods are described in the preceding paper.

Results are listed in Table I of the preceding paper.

Discussion: When ovaries were transplanted to the spleen, a marked hypertrophy occurred (normal, 49.9 mg., transplant, 149.5 mg.). This could be due to increased production of pituitary gonadotrophin or to decrease in circulating estrogen reaching the ovaries. Assay of gonadotrophin content of the anterior pituitary glands of the transplant animals revealed relatively normal gonadotrophin levels (normal 10.3 mg., transplant, 30.8 mg.) militating against the possibility of increased gonadotrophin stimulation. The hypothesis of decrease in circulating estrogens (removal of inhibition by estrogen) is favored by the 300% increase in ovarian weights noted in the autotransplants to the spleen. It is unlikely that the slight rise in pituitary gonadotrophic content accounts for the increase in ovarian weight, since the adhesion control rats developed similar gonadotrophic potency without similar ovarian growth. The inhibition of ovarian growth in the autotransplant rats with vascular adhesions to the systemic circuit must have been due to the presence of circulating estrogen in the blood.

To confirm this observation, two sets of animals were injected

subcutaneously daily for 34 days with estradiol-benzoate. Group I received from 0.5 γg to 5.0 γg per day. These doses were greater than normally required by the animals, judged by the decrease in thymus weight from 154 mg. to 132 mg., and the estrus condition of the uterus. Ovarian weights were markedly suppressed by the injections. The average at operation was 62.3 mg. and after injection (at autopsy) 14.9 mg. Suppression of pituitary gonadotrophic potency to below normal was noted.

A second group was injected with 0.05 γg to 0.35 γg of estradiol benzoate daily. This lower dosage was more physiological, as judged by the normal weights and appearance of the thymus and uterus. However, not only was the 300% increase in ovarian weight prevented but actual atrophy occurred. The ovarian weights fell from 46.0 mg. at operation to 21.5 mg. at autopsy. This suppression in ovarian weight is not likely due to estrogen inhibition via the pituitary gland but due to direct inhibition of the ovary, since pituitary potency did not drop. In fact, as could be expected from the decrease in ovarian activity, the pituitary potency actually rose above normal during the period of estrogen administration.

While the presence of circulating pituitary gonadotrophin is the sine qua non of ovarian stimulation, in the presence of adequate amounts ovarian activity seems to largely be regulated by the level of circulating estrogens present.

Summary

Both ovaries of mature female rats were autotransplanted into the spleen, allowing the ovaries to be stimulated by pituitary gonadotrophins, but denying them the presence of circulating estrogen by

interposing the liver between the ovaries and the systemic circulation.

The ovaries of these animals increased threefold in weight after 30-57 days transplantation (149.5 mg.) over their weight at the time of operation (49.0 mg.).

Two groups of experimental animals were injected with estradiol benzoate. One group received amounts exceeding physiological requirements and the other amounts approximately meeting physiological requirements. In both groups, the ovarian weights showed a marked decrease below the weight at operation.

It is concluded that ovarian growth is inhibited by the presence of circulating estrogens.

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