

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 μ g) 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 μ g 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 μ g) 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.

15894

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

CARL G. HELLER AND EDWIN C. JUNGCK.

From the Department of Physiology, University of Oregon Medical School, Portland, Oregon.

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

Heller, Heller and Sevringhaus¹ 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.²

Control of ovarian growth and secretion in a normal intact animal may be by either (or both) of 2 mechanisms: (a) Stimulation of growth by pituitary gonadotrophin secretion (at position 1 in Fig. 1, preceding paper) and (b) active suppression of growth by estrogenic inhibition (at position 3 in Fig. 1, preceding paper).

To study this problem, a preparation must be devised which will separate these 2 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, 20.4 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 μ g to 5.0 μ g 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 μ g to 0.25 μ g 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 48.0 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.

¹ Heller, C. G., Heller, E. J., and Sevringhaus, E. L., *Endocrin.*, 1942, **30**, 309.

² Galli-Mainini, C., *Endocrin.*, 1941, **29**, 674.

Whereas 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

3-fold 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.

15895

Bodies Suggesting Exoerythrocytic Forms of *Plasmodium vivax* in Tissue Culture.*

I. N. DUBIN. (Introduced by Douglas H. Sprunt.)

From the Division of Pathology and Bacteriology, University of Tennessee College of Medicine, Memphis.

The demonstration of exoerythrocytic forms of avian malarial parasites¹ has stimulated a search for similar bodies in mammalian malaria. A few authors have described structures which they believed to be exoerythrocytic forms of human malarial parasites. The subject has been reviewed by Porter and Huff² and by Brug.³ Brug³ himself presented figures of bodies seen in smears of the lungs of a patient dying in the acute stage of *Plasmodium vivax* infection which he thought to be exoerythrocytic forms. Recently Raffaele⁴ has reiterated the claim that he has seen such forms in all 3 types of human malaria.

To date, demonstration of exoerythrocytic forms has depended largely upon examination of smears and sections of tissues. Recently Hawking^{5,6} has shown that the exoerythrocytic forms of avian malarial parasites are readily grown in tissue cultures of infected tissues. This has been confirmed for *P. gallinaceum* by Haas,⁷ Zuckerman⁸ and by the present author.⁹ Since methods of tissue culture allow for multiplication of the histotropic forms of the parasites, this technic might prove superior to the use of smears or sections in the search for mammalian exoerythrocytic forms. Accordingly we have made tissue cultures of bone marrow from humans infected with sporozoites of *P. vivax* or *P. falciparum* both before and after parasites

* This work was supported in part by a grant-in-aid from the Tennessee Valley Authority.

¹ Huff, C. G., and Bloom, W., *J. Infect. Dis.*, 1935, **57**, 315.

² Porter, R. J., and Huff, C. G., *Am. J. Trop. Med.*, 1940, **20**, 869.

³ Brug, S. L., *Nederl. tijdschr. v. geneesk.*, 1941, **84**, 2745.

⁴ Raffaele, G., *Trop. Dis. Bull.*, 1946, **43**, 1016 (abstract).

⁵ Hawking, F., *Trans. Roy. Soc. Trop. Med. and Hyg.*, 1945, **39**, 245.

⁶ Hawking, F., *Trans. Roy. Soc. Trop. Med. and Hyg.*, 1946, **40**, 183.

⁷ Haas, V. H., personal communication, 1946.

⁸ Zuckerman, A., *J. Infect. Dis.*, 1946, **79**, 1.

⁹ Dubin, I. N., in preparation.