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Effects of Pituitary Removal or Transplantation on Ovarian Ascorbic Acid Depletion in the Rat.* (26339)

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It is now accepted that luteinizing hormone of the pituitary (LH), when given intravenously to suitably prepared immature rats, will deplete ovarian ascorbic acid. This procedure forms the basis for a sensitive assay method for LH(1) and it has been reported that the other anterior lobe hormones are without effect on this response(1,2). Briefly, the assay animals are prepared by treating 25-26-day-old female rats with exogenous gonadotrophins to induce precocious ovulation and obtain animals with heavily luteinized ovaries. It has been shown previously that, following ovulation, such animals remain in a state of pseudopregnancy for 10-12 days as judged by vaginal smears, decidual reactions and the Hooker-Forbes test for plasma progesterone(3). Furthermore, it must be presumed that luteal function in these immature animals is promoted by pituitary luteotrophin secretion. It is not known, however, to what extent the ovarian response to LH (ascorbic acid depletion) may be conditioned or modified by other circulating pituitary hormones. It has been reported that a rapid loss of ovarian ascorbic acid follows hypophysectomy and that this is attended by a decreased sensitivity to LH (4). Since it has been shown that pituitary autografts in the kidney are capable of maintaining luteal function in the adult rat(5) it seemed of interest to study the response of ovarian ascorbic acid to LH in immature, pseudopregnant rats after hypophysectomy and hypophysectomy followed by grafting the pituitary under the renal capsule. In the latter situation it was anticipated that a continuous luteotrophin secretion by the graft would maintain the corpora lutea functional for extended periods of time. At the same time the ovaries of the autograft-bearing rats would be removed from any endogenous source of follicle stimulating hormone (FSH) or luteinizing hormone (LH) since it has been found that pituitary grafts in the kidney are incapable of secreting these hormones(5,6).

Methods. Holtzman female rats, 25 days of age, were injected (sbc) with 50 IU of pregnant mares' serum gonadotropin (Equinex).§ This was followed by an injection

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TABLE I. Changes in Ovarian Weight and Ascorbic Acid Concentration in Immature, Pseudopregnant Rat* Following Hypophysectomy or Pituitary Autografting.

Animal group	Treatment		No. of rats	Ovarian wt (mg) †	Ascorbic acid (mg/100 g)
1	Intact, pseudopregnant 6 days		24	115.8 ± 4.5‡	85.7 ± 5.0
2	Pseudopregnant 6 days + hypex.	1 wk	16	85.6 ± 5.1	85.1 ± 4.9
3	*Idem*	2 "	7	95.8 ± 5.1	92.8 ± 6.3
4	"	4 "	14	84.0 ± 4.6	71.6 ± 4.3
5	"	5 "	13	69.7 ± 3.3	85.4 ± 2.9
6	Pseudopregnant 6 days + autografts	1 "	15	117.9 ± 6.6	89.4 ± 5.2
7	*Idem*	2 "	8	123.1 ± 6.7	78.6 ± 7.0
8	"	4 "	15	102.3 ±10.3	58.2 ± 3.2

* All animals were made pseudopregnant by inj. of PMS and HCG.
† Single ovary wt.
‡ Stand. error.

of 25 IU of human chorionic gonadotropin‖ (HCG) 64 hours later. Six days after HCG injection some of the rats were studied as intact, pseudopregnant animals and others were hypophysectomized. The hypophysectomy operation was done by the usual parapharyngeal approach using ether anesthesia and without tracheal cannulation. In several groups of animals the pituitary was removed and quickly autotransplanted to the renal capsule. Following surgery the animals were given a 5% solution of dextrose to drink and the routine diet of Purina laboratory chow was supplemented at intervals with fresh carrots and lettuce. The first objective was to determine the changes with time after operation in ovarian weight and ascorbic acid level. These data are shown in Table I. At autopsy the ovaries were removed, dissected free of uterine tube and connective tissue and weighed on a torsion balance. Ascorbic acid analyses were carried out by a scaled up version of the micromethod described by Lowry *et al.*(7). In other groups of animals prepared in the same manner the influence of hypophysectomy or pituitary autografting on responsiveness of ovarian ascorbic acid to LH depletion was studied (Table II). Four hours prior to autopsy the animals were anesthetized with ether and the left ovary was removed surgically. A standard dose of LH¶ (3.2 μg/100 g) was then injected into a tail vein. Preliminary studies in our laboratory had shown that this dose of LH would induce a maximal depletion of ovarian ascorbic acid when given to pseudopregnant rats in accordance with the conditions of the Parlow assay. At autopsy of these animals the right ovary was removed and prepared for ascorbic acid analysis. In all hypophysectomized animals the sellar region was examined carefully for pituitary remnants. Body weight records were also available and observations of hair texture were made(8). Animals were discarded if evidence suggesting incomplete removal of the pituitary gland was found. In 3 groups of animals studied (Table II, groups 2, 6, 9) one ovary had been removed prior to their use in this study so that no "before LH" ascorbic acid value is shown. Except for these 3 groups the change in ovarian ascorbic acid produced by LH injection is represented in Table II as "% change." In all cases where it was considered desirable to compare mean differences the standard "t" test for significance was applied.

Results. The results are shown in Tables I and II. For the 4 week period studied the ovarian weight is well maintained by the pituitary autografts (Table I). There is, by

‖ Kindly supplied by Dr. L. T. Blouin, Parke Davis and Co., Ann Arbor, Mich.

¶ This preparation was kindly supplied by Prof. C. H. Li, Hormone Research Lab., Berkeley, Calif. It carried the designation Sheep ICSH but because of the nature of this study we prefer to use the alternative designation of Luteinizing Hormone (LH).

TABLE II. Effect of Hypophysectomy or Pituitary Autografting on Ovarian Ascorbic Acid Depletion by LH in Immature, Pseudopregnant Rat.*

Animal group	Treatment		No. of rats	Ovarian ascorbic acid (mg/100 g) Before LH†	After LH‡	% change
1	Intact, pseudopregnant 6 days		7	88.8 ± 4.8§	46.1 ± 4.7	−48
2	Pseudopregnant 6 days + hypex.	1 wk	8	—	101.9 ± 9.0	—
3	*Idem*	1 "	9	81.4 ± 5.6	88.6 ± 7.8	+ 8.8
4	"	4 "	7	78.7 ± 4.7	76.3 ± 4.6	− 3.0
5	"	5 "	7	87.0 ± 4.0	90.1 ± 5.9	+ 3.6
6	"	5 "	6	—	83.7 ± 3.9	—
7	Pseudopregnant 6 days + autografts	1 "	7	83.3 ± 4.2	31.4 ± 5.1	−62
8	*Idem*	4 "	7	61.0 ± 4.3	30.1 ± 3.0	−51
9	"	5 "	9	—	30.9 ± 3.0	—

* All animals were made pseudopregnant by inj. of PMS and HCG.
† Left ovary was removed surgically under ether anesthesia just prior to inj. of 3.2 μg LH/100 g body wt via tail vein.
‡ Right ovary was removed 4 hr after LH inj.
§ Stand. error.

contrast, a significant decrease in ovarian weight following hypophysectomy. Less pronounced differences are seen in concentration of ovarian ascorbic acid in the various groups. Statistically, the only significant decrease in ovarian ascorbic acid level ($P = < .001$) occurred in those animals (Table I, group 8) which had pituitary autografts for 4 weeks.

A dramatic difference in responsiveness of ovarian ascorbic acid to LH is seen when the effect of hypophysectomy is compared to pituitary autografting (Table II). When given to animals hypophysectomized for periods of from one to 5 weeks LH was without significant effect on ovarian ascorbic acid (Table II, groups 2-6). The same dosage of LH given to animals bearing pituitary autografts led to depletion of ovarian ascorbic acid even greater than that obtained in intact, pseudopregnant rats (Table II, groups 7-9). The depletion seen in the intact and autograft bearing rats is, in all cases, highly significant ($P = < .001$).

Discussion. It has long been known that corpora lutea will remain in ovaries of adult rats for long periods after hypophysectomy (9). Such corpora lutea are physiologically inactive, however, in contrast to the functional corpora lutea which can be maintained for months in the adult rat by pituitary autografts(5). We have found that pituitary autografts in immature animals as used in the present study are incapable of maintaining ovarian weight for periods longer than 4 weeks (unpublished observations). Maintenance of ovarian weight is remarkably good for 4 weeks after operation although a significant decline in ovarian ascorbic acid level is already apparent (Table I).

The difference in response of ovarian ascorbic acid to LH in hypophysectomized animals as compared to intact or autograft-bearing rats is an important one. It indicates that, for LH to deplete ovarian ascorbic acid, the ovary must be under the influence of luteotrophin. This explains the rapid decline in ovarian ascorbic acid and the decrease in its sensitivity to LH after hypophysectomy as reported by McCann *et al.*(4). We also have found that a significant fall in concentration of ovarian ascorbic acid takes place during the first 24 hours after hypophysectomy. A return to a value not different from the level in the intact, pseudopregnant animal then occurs. It now seems likely that this initial decrease which follows hypophysectomy may be due to a release of LH from the pituitary during its removal. Whether or not this is the case, it is now obvious that hypophysectomized animals are basically unsuitable for assay of LH by the ascorbic acid depletion method. On the other hand, animals made pseudopregnant in the usual manner and subjected to the pituitary autografting procedure should prove useful in several

ways. Since such grafts appear incapable of secreting detectable amounts of either FSH or LH the problem of possible interference by endogenous LH in ovarian ascorbic acid depletion is removed. Likewise, in these animals the possibility of ovarian ascorbic acid depletion being due in part to LH-releasing factors as opposed to LH *per se* is eliminated. For these reasons, it would seem desirable to utilize such animals in conjunction with the usual assay animal in testing various agents and biological materials for LH-releasing substances. In this connection there is disagreement in the literature as to whether vasopressin and adrenaline will affect ovarian ascorbic acid in the hypophysectomized animal(2,4). Finally, it should be emphasized that for routine assay of LH we consider the procedure described by Parlow(1) using intact, pseudopregnant animals to be quite satisfactory. It will of course, be necessary to use intact animals as test objects in further search for LH-releasing substances.

Summary. Immature female rats were made pseudopregnant in the manner prescribed for the Parlow LH assay. Hypophysectomy of such animals on the sixth day of pseudopregnancy caused a gradual decline in ovarian weight over a 5-week period but concentration of ovarian ascorbic acid was well maintained. At periods of a week or more after hypophysectomy intravenous injection of LH produced no decline in ovarian ascorbic acid. In other animals the pituitary gland was autografted to the renal capsule on sixth day of pseudopregnancy. Ovarian weight was well maintained in these animals for 4 weeks although at 4 weeks there was a significant decrease in level of ovarian ascorbic acid. Intravenous injection of LH in pituitary graft-bearing rats led to depletion of ovarian ascorbic acid even greater than that found in intact, pseudopregnant rats. It is suggested that animals bearing pituitary autografts should prove valuable in the search for LH releasing factors.

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Studies of Serum Protein Abnormalities in Kala Azar.* (26340)

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Patients with kala azar have long been known to have hyperglobulinemia. Recently, moving boundary and filter paper electrophoresis of serum proteins from such patients have shown a nonspecific diffuse hypergammaglobulinemia and hypoalbuminemia without significant alterations in alpha or beta globulins(1,2,3,4,5). A few workers, however, have reported what they believed to be specific abnormalities. Benhamou *et al.*(6)

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