Effects of Sibling and Nonsibling Pituitary Grafts and Ovine Luteotropic Hormone in Long-Evans Female Rats[1] (36548)

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Ever since successful functional restitution of pituitary iso- and homografts in hypophysectomized male and female recipients was reported (1) a number of studies utilizing multiple (from 2–27) donor pituitary grafts have shown the ability of ectopically placed pituitaries to secrete virtually all of the tropic hormones in hypophysectomized rats (1–6). In spite of rather convincing warning by Kendall and Allen (7) regarding acceptance of grafts as well as suitability of a particular strain of rats for a given physiological study, the use of transplanted rats is indiscriminate for the most part. In terms of acceptability of pituitary grafts in various strains of rats there is a great deal of variability and in some instance discrepancy in the literature. In studies on the effects of pituitary grafts on the estrous cycle of intact Sprague-Dawley rats approximately 59% of the transplants became functional (8), and all of the intraocularly placed grafts were accepted in Charles-River strain of rats (9). Smith and Davidson (5) using Long-Evans rats reportedly did not encounter any rejection of grafts, however, they noted positive effects in only 8% of their rats. In a study (10) utilizing intact Long-Evans female rats grafted with pituitaries from male and female donors, we noted that those rats displaying prolonged periods of diestrus had been grafted with sibling pituitaries and those returning to normal estrous cycle had received nonsibling grafts. In order to verify these earlier observations; and also to estimate the amount of exogenous mammotropin (MH, LtH, prolactin) necessary in the Long-Evans rat to imitate the graft in interfering with the estrous cycle [see Lahr and Riddle (11) and Anderson (12)] the following experiments were undertaken.

Methods and Materials. All of the rats studied in these experiments were Long-Evans virgins, 3–5 months of age. The donor rats were from the same colony, of the same age and included males as well as females. Whole pituitaries were placed under the capsule of the left kidney, except in some of the autografted animals which usually received the pituitary in halves. In the hypophysectomized rats, the grafts were implanted while the animal was etherized for the ablation. Vaginal smears were studied daily throughout the experiments. The duration of experiments varied from 46 to 90 days and is reported in the Results section for different groups.

At necropsy, the ovaries, uteri, mammary glands, and grafts were fixed in Bouin's fluid or 10% formalin; and were then processed for histologic study. Tabulations were made of body weight changes, histologic evaluation of the corpora lutea, uteri and mammary glands. Arbitrary ratings on a 1+ to 4+ scale were assigned to the grafts. The 4+ grafts were so rated because of their size, good vascularity, minimal necrosis, fibrosis and infiltration and the "healthy" appearance of the cells stained with the periodic acid–Schiff, orange-G, and hematoxylin method.

Seven different groups of rats were used and are reported as follows:

1. 9 female nonsibling grafts in intact females;

2. 32 sibling (15 male and 17 female)

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grafts in intact females;

3. 8 nonsibling female grafts in hypophysectomized females;

4. 26 sibling grafts (10 male and 16 female) in hypophysectomized females;

5. 5 autografts in hypophysectomized females;

6. 4 control hypophysectomized rats receiving no treatment;

7. 18 intact females injected with 3 different levels of ovine MH, and 6 normal controls that received saline.

Results. Expt. 1. Nonsibling female grafts in normal adult females. Eight of the 9 rats studied for 50–62 days showed a suspension of the regular estrous cycle (8–14 days) as in pseudopregnancy and then returned to normal estrous cycles. One rat showed no interruption in its estrous cycles; and showed no evidence of a graft at necropsy. In 8, the implants were small and in various stages of rejection (Fig. 1). They were infiltrated with lymphocytes and macrophages, and organized by fibroblasts. A few, questionably viable, parenchymal cells showed nuclear pyknosis. The target organs' morphology was commensurate with the stage of the estrous cycle at the time of autopsy.

Expt. 2. Male and female sibling grafts in normal adult females. In 3 of 15 rats with grafts from male siblings (duration of experiment 71 to 90 days) and 5 of 17 with grafts from female siblings (duration of experiment 47 to 68 days) transplants were not recovered at autopsy. From the remaining rats the transplants recovered from the kidney were sectioned and rated on the basis of their histological appearance and likely viability. Both male and female grafts were accepted equally well. Based on the criteria outlined in the Methods section, 13 of the 24 accepted grafts were rated as 4+, 6 as 3+, 2 as 2+ and 3 as 1+. Eight rats in which the grafts were not located at autopsy and 1 with the graft rated as 3+, returned to normal estrous cycles within the first 2 weeks of transplantation. Eight rats with morphologically viable grafts (2 = 1+, 2 = 2+, 3 = 3+ and 1 = 4+) returned to normal estrous cycles 20 to 30 days prior to necropsy.

In most rats the luteotropic effect began immediately as shown by inhibition of a fully

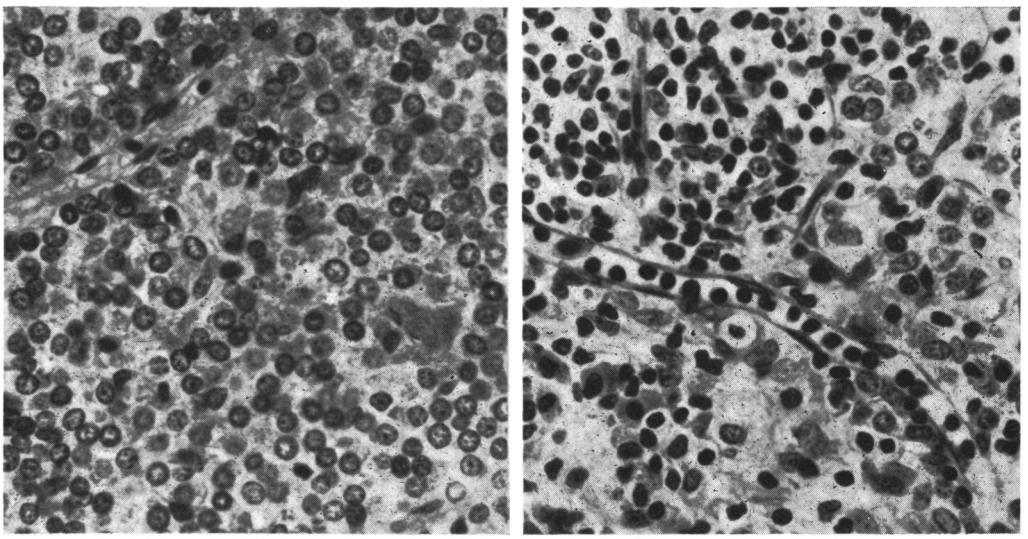

FIG. 1. (left) Photomicrograph of a functionally and morphologically viable pituitary graft from a male sibling rat implanted below the renal capsule of an intact, adult female. This rat showed no cornification of the vagina for the duration of experiment (71 days). ×330. (right) Photomicrograph of a rejected pituitary graft from a nonsibling female rat implanted below the renal capsule of an intact adult female. After an initial period of 2 weeks of diestrus vaginal smears, this rat showed normal estrous cycles for the remainder of the experiment (66 days). ×330.

cornified vaginal smear extended over different periods. The time of the appearance of the next typical cornified smear after grafting varied from 2 to over 71 days. At one extreme were 3 rats that showed no cornification for the entire graft period of 71 days. Their grafts were in excellent condition at necropsy (Fig. 1). However, these and all other rats with prolonged periods of diestrous smears between cornified vaginal states did show vaginal mucification at the interval of approximately 11 days, which was not followed by cornified smears. These periods of mucification in which the round mucin-secreting epithelial cells are abundant in the vaginal smears may have represented behavioral "heat"; but the rats were not tested with males. In other instances of prolonged diestrous states cornified smears were usually preceded by a mucified smear. Fifteen rats with viable grafts and prolonged periods of diestrus showed functional hypertrophy of the latest crop of corpora lutea. Four of the 8 rats which had morphologically viable grafts but had returned to normal estrous cycles during the last 20 to 30 days of the experiment, also showed luteal stimulation. The ovaries of the remaining 13 rats were comparable to that of a normally cycling rat.

Rats bearing male and female viable grafts displaying prolonged periods of diestrous smears interrupted by mucified and or cornified smears showed lobulo–alveolar mammary growth which is indicative of a previous or continuing influence of as estrogen plus a progestogen and the mammotropic hormone. Rats without grafts and others with grafts which had returned to regular estrous cycles 20 to 30 days prior to necropsy showed what would be expected, namely, a cyclic type of mammary gland with ducts and alveolar buds which sometimes form small clusters.

Expt. 3. Nonsibling female grafts in hypophysectomized rats. In the 8 rats of this group, the body weight loss averaged 28 g in the rats sacrificed 48 days after hypophysectomy, and 46 g for the 62-day postoperative animals. All of the vaginal smears from the second day after hypophysectomy until necropsy showed scanty leukocytes and desquamating epithelial cells typical of diestrus. The grafts or the graft areas were fibrotic and infiltrated with lymphocytes and macrophages. An occasional parenchymal cell that may have been viable was observed. There was no evidence in the corpora lutea that the graft had been releasing luteotropic hormone just prior to necropsy. The latest crop of corpora lutea was present in different stages of maintenance and degeneration, but not stimulation. The uteri and mammary glands were atrophic; and the ovaries resembled those of the hypophysectomized untreated controls.

Expt. 4. Male and female sibling grafts in hypophysectomized females. Of the 26 rats studied for 46 to 66 days, 10 received male and 16 female sibling grafts. Three of the rats with male sibling grafts and 1 of the rats with female sibling grafts were rejected. The size and appearance of the accepted grafts as seen at necropsy, and on histologic examination, varied considerably (7 grafts rated 4+; 3 = 3+; 8 = 2+ and 4 = 1+). Regardless of the stage of the estrous cycle at the time of hypophysectomy and homografting, at necropsy the vaginal smears and the vaginal histology on section in all cases were of the diestrous type (*i.e.*, consisting of a few leukocytes and desquamating, squamous, epithelial cells). As judged by the appearance of the corpora lutea and the uterine epithelium, 12 of the 22 grafts had released luteotropic hormone; and the corpora lutea had secreted some progestogen. The mammary glands showed ducts with sparse alveolar buds; and in no case was lobulo–alveolar development seen, thus providing evidence against estrogen secretion by the ovaries and FSH-ICSH secretion by the graft. This was also supported by the absence of the cornified or mucified type of vaginal smears throughout the experiment.

Expt. 5. Autografts in hypophysectomized rats. All of the 5 transplanted pituitaries were maintained for the duration of 48 days. The rats lost an average of 21 g in weight, thus providing no evidence of any appreciable secretion of STH by the grafts. The ovarian interstitial tissue and follicles, the vaginae and mammary glands were regressed or atrophic; and these findings led to the conclusion

that the grafts were not secreting ICSH or FSH. Two of the rats showed good luteal cell stimulation, 2 showed the usual maintenance of corpora lutea in hypophysectomized rats and also showed signs of slight stimulation (functional hypertrophy).

Expt. 6. Untreated hypophysectomized rats. Three of these 4 rats hypophysectomized in proestrus showed successively a mucified and then a cornified smear typical of a continuing cycle. For the remaining 46 days, the cycle did not recur and all of the smears were of the scanty "leukocytes and epithelium" type. The fourth animal hypophysectomized on the day following cornification showed the same diestrus type of smear without a recurrence of cornification. Histologic study of the ovaries, uteri, vaginae and mammary glands revealed no evidence of any hormonal stimulation. The latest set of corpora lutea, in each case, showed good maintenance but no evidence of stimulation (*i.e.,* functional luteal cell hypertrophy). Two earlier sets of corpora lutea showed considerable degeneration and shrinkage.

Expt. 7. Normal adult female rats injected with ovine mammotropin. In the rats injected daily for 1 month with 3 levels of ovine MH the results were as follows: at 15 IU daily, cornification reappeared after periods of 6 to 20 days in all 6 rats, and essentially normal cycles followed; at 30 IU no cornification was observed in 3 rats throughout the month of observation; and in 3 it recurred on day 16, 19, and 23, respectively; at 60 IU no cornification was seen in 2 during the month; and in 4 it reappeared on day 16, 16, 28, and 30, respectively. Most of the rats showed a reappearance of a mucified smear comparable to that of the precornification stage of the normal cycle. The mucified smear was considered indicative of a combined estrogen–progestogen action on the vagina. The absence of cornification on the following day was thought to be due to a higher progestogen–estrogen ratio than is normally consistent with cornification. Each of the 6 saline injected control rats showed regular 4–5 day estrous cycles.

Discussion. Rat anterior pituitary when transplanted to a distant site has been shown to secrete MH (13) and may be implicated in the secretion of other tropic hormones (2–6). In terms of acceptance of a transplant, an autografted pituitary presents no difficulty; however, this is not uniformly true for homografts. Kendall and Allen (7) using adrenal weights as an end point in their study found some variability in the adrenal weights in various strains of homografted hypophysectomized rats. However, in Long-Evans rats of our colony, randomly selected nonsibling pituitary grafts of males and females were rejected by the normal as well as hypophysectomized female hosts. Even the sibling male and female donated grafts were not accepted by all of the recipients. Moreover, all of the rats which showed morphologically viable sibling grafts did not respond uniformly. There was a wide spectrum of responses to a single sibling pituitary graft in the intact females. The main tendency was towards interruption of the regular estrous cycle and the substitution in some cases of "pseudopregnancy type" cycles of varying length. Some rats showed regular short (4–5 day) cycles with recurring mucification but not cornification. One rat in Expt. 2 with a female sibling graft rated 3+ after 47 days, continued throughout the experiment to show regular cornification cycles of 4 to 5 days. It is of interest to note that Smith and Davidson (5) using the same strain of rats did not report any rejection of donor pituitaries in the hypophysectomized male hosts, even though 92% of their experimental rats did not show spermatogenesis.

The effect of a functionally viable pituitary graft superimposed on a rat's own pituitary was that of augmenting the life of the newest generation of corpora lutea by a continuous secretion of luteotropic hormone and thereby creating a state comparable to pseudopregnancy as was shown earlier (8, 9). Even though the production of MH may have been continuous (14, 15) the phase of pseudopregnancy did not last indefinitely. Evidently there was enough production of FSH and ICSH by the *in situ* pituitary, and therefore of estrogen from the ovaries to elicit mucified and at times cornified vaginal smears.

The once-daily administered ovine MH to intact rats did not precisely imitate the continuous secretion of MH by the grafted pituitaries. Moreover, one might suspect that a portion of the ovine MH was being inactivated by the antibodies to the foreign protein. Nevertheless, some of the rats with apparently viable grafts showed no more luteotropic effect than those on 0.5 mg (15 IU) of ovine MH. The 1 mg (30 IU) and 2 mg (60 IU) doses were effective to a degree quite comparable to that of many rats with functionally viable grafts. (*i.e.*, there was no return of vaginal cornification for the duration of the experiments). Even though there is a likelihood that the injected MH as well as that from grafts may have inhibited the MH of a rat's own pituitary, the FSH-ICSH was not totally inhibited by any of the treatments as evidenced by breakthrough mucified and/or cornified smears. The secretion of FSH-ICSH is attributed to the *in situ* pituitary because hypophysectomized rats transplanted with viable sibling grafts or autografts showed no evidence of follicular and interstitial stimulation, or estrogen secretion.

Summary. Pituitary autografts and sibling and nonsibling homografts placed under the renal capsule were studied in normal and hypophysectomized rats. In these Long-Evans rats, which were not closely inbred, grafts from nonsiblings were rejected; as were about 20% of those from siblings. Most of the accepted grafts secreted LtH throughout the experiment (up to 3 months) as shown by functional luteal cell hypertrophy and progestogen secretion. In order to estimate the approximate amount of exogenous hormone required to imitate the estrous-cycle-inhibiting effect of these grafts purified ovine mammotropin with 30 IU/mg was used. With due consideration for the considerable variability in the responsiveness of our rats to this hormone, as well as in the secretory activity of a transplanted pituitary, it might be concluded that 15 to 60 IU of the hormone covers the range of LtH activity exhibited by single viable pituitary grafts.

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