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Received May 23, 1966. P.S.E.B.M., 1966, v123.

Reestablishment of Ovarian Periodicity After Transplantation to the Syrian Hamster Cheek Pouch.* (31540)

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Homologous and autologous ovarian transplantation has been successfully accomplished in a variety of mammalian species. Sites have varied from orthotopic to general abdominal, to tail, ear, leg, and anterior chamber of the eye(1). Homologous orthotopic transplantation reestablished limited fertility in mice(2), and apparently normal cyclic ovarian periodicity in several other species for short durations. Newly established ovarian grafts respond characteristically to gonadotropins, may resume normal steroid synthesis for a short time, but soon begin to secrete excessive quantities of androgen after location in these peripheral sites. Under these conditions cyclic activity comes to a halt and investigation is limited to short-term experiments. In each of the grafting sites mentioned there also appears a limitation either of restricting the size which the graft may attain or difficulty in making direct observation of the transplant. The cheek pouch of the Syrian hamster *(Mesocricetus auratus)* provides excellent opportunity for both tissue growth and visual inspection of the graft throughout extended investigation. Results of our experience in transplanting ovaries to this site are reported herein.

The pouch was first used in 1951 for transplantation of neoplastic tissue(3) but more recently normal pancreas(4), oviduct(5), and uterus(6) have been accepted by this highly vascular area. Homologous grafts may persist indefinitely, and tissues from rat and rabbit continue to be supported for weeks. Even human tissue transplants have been maintained in the cheek-pouch for at least 45 days. As a partial explanation for the unique suitability of the pouch for transplantation studies, Shepro(7) has pointed to its alymphatic nature and has suggested that outward diffusion of graft antigens may be slowed by the large quantity of dense connective tissue that makes up a major portion of the pouch's inter-membranous space.

Methods. Sexually mature female hamsters, weighing between 55 and 85 g were anesthetized (Nembutal intraperitoneally, 7 mg/100 g body wt.) and bilaterally ovariectomized through a mid-ventral incision. The left pouch of each animal was everted and pinned onto a cork-covered operating board. A variable light source illuminated the pouch as it was stretched over a hole in the board, allowing the investigator to properly position

* Contribution No. 391, New Hampshire Agr. Exp. Station. Supported by NIH grant HD-00752 and regional NE-41 (Hatch Act) funds.

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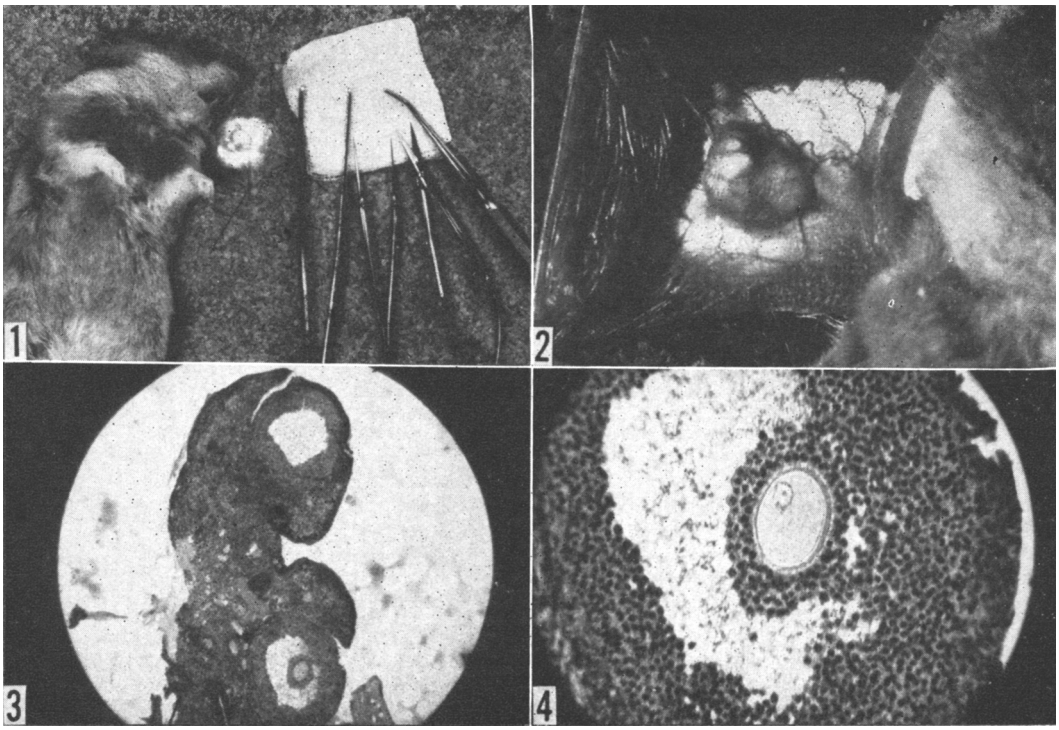

FIG. 1. Technique for ovarian transplantation.
FIG. 2. Successful ovarian homotransplant.
FIG. 3. Transplanted ovary 35×.
FIG. 4. Tranplanted ovary 440×.

the tissue, and to examine the implant whenever desired (Fig. 1). Using iris scissors, an incision was made in the outer epidermis just large enough to permit passage of the excised ovary. By carefully probing an area near the bifurcation of the major vessel that supplies the pouch and placing the implant at this site, the greatest degree of implant acceptance was obtained. Vascularization was usually evident by 5 to 7 days and well-advanced at 11 days post-insertion. A single suture was usually sufficient to close the incision. The surface was then swabbed with 70% ethanol prior to reinserting the pouch to help prevent infection.

Repeated observation (as often as twice daily for 5 days) in several animals showed no noticeable deleterious effect to either host animal or implant. Animals could be maintained in an anesthetized state for over an hour using repeated injections of Nembutal as necessary, as long as the everted pouch was moistened frequently. Duby and McDaniel (8) caution that repeated use of Nembutal may interfere with ovulation time.

Selected successful ovarian transplants were removed, sectioned at 8-10 micra, stained in hematoxylin and eosin, and compared with appropriate controls. Estrous cycles were determined using the characteristic lordosis reflex behavior as evidence of day 1. Experimental animals were mated and subsequent pseudopregnant cycles measured as further evidence of the transplant's ability to regulate sexual periodicity from the new site.

Results. Normal ovarian periodicity was successfully reestablished in 16 of 30 ovariectomized animals in which ovaries were transplanted to the cheek pouch (Table I). As criteria for success, both 4-day estrous and 9- to 10-day pseudopregnant cycles were measured for at least one month. A successful transplant is shown in Fig. 2. Comparative histological examination of explanted ovaries revealed that the grafts had retained their

TABLE I. Cycle Length in Normal and Experimental Hamsters.

No. animals	No. cycles	Ovary position	Cycle length (days) Estrous	Pseudo-pregnant
10	59	Normal	4	9.24 ± .16*
16	85	Cheek pouch	4	9.85 ± .28

* Mean ± standard error.

characteristic morphology (Fig. 3, 4). A closer examination of the follicles produced by transplanted ovaries showed no visible evidence of morphological alteration, leading us to believe that subsequent studies on ovulation might be profitable using this technique.

It should be mentioned that there was a tendency for implants to enlarge and produce "cystic-like" follicles after several normal cycles in the pouch. Some implants have successfully regulated estrous and pseudo-pregnant cycles for as long as 6 months, only to show eventually the same loss of cyclic function due to rapid follicular enlargement.

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Received June 16, 1966. P.S.E.B.M., 1966, v123.

Effect of 1-Methyl-2-Mercaptomidazole (Methimazole-Tapazole) on the DNA Content of the Rat Thyroid Gland.* (31541)

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In the estimation of thyroid hormone secretion rate (TSR), the goitrogen methimazole or tapazole has been used to block the recycling of I^{131} from the metabolism of L-thyroxine-I^{131}. This goitrogen has been used since it has been shown to have no extrathyroidal effect in comparison to other goitrogens (1). In the rat, it has been shown that 400 μg/100 g b.w./day by subcutaneous injection will block I^{131} recycling(2). After 30 days of treatment, it has been shown that the rat thyroid gland doubles in weight. Histological study of the thyroid glands indicates that the goitrogens deplete the gland follicles of all stored colloid but greatly increase the height of the follicular epithelium. Since the glands become dark red in color, it may be assumed that there is an increase in the capillary bed and blood content. It is not clear whether the doubling of the gland weight is primarily due to an increase in cell multiplication due to TSH or to an increase in cell size. If it were due to cell multiplication, such glands might be capable of greater thyroglobulin secretion when the goitrogen was withdrawn.

Matovinovic and Vickery(8) fed male guinea pigs 0.2% thiouracil in the feed for a period of 3 months. They reported 314% increase in thyroid weight, a 519% increase in total cell mass, a 140% increase in average cell height and with no change in cell population density. The DNA per unit weight of wet tissue was not changed while the DNA per unit weight of cell mass decreased 36%. The total DNA per gland increased 299%.

* Contribution Missouri Agr. Exp. Sta. Journal Series 4004. Approved by Director. This investigation was supported in part by a grant from U. S. Atomic Energy Commission Contract AT(11-1)-301-125.