However, the effects of dietary fats are largely or wholly achieved in a few weeks, with little or no subsequent change.

Summary. Rigidly controlled experiments on middle-aged men subsisting on diets of natural foods with and without supplements of 15 g daily of either cellulose (fiber) or pectin failed to show any significant effect on serum cholesterol concentration from the cellulose but they did consistently show an effect from the pectin. The pectin effect was apparent in 3 weeks and amounted to an average fall of about 5% below the level on the same diet without pectin supplement. It is suggested that the amounts of cellulose and pectin used correspond to the upper levels of these substances provided in natural human diets.

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Gonadotropin Secretion in Lactating Mice.* (26402)

W. A. SADLER AND H. C. BROWNING

Department of Anatomy, University of Texas Dental Branch, Houston, Texas

Ovariectomized mice, bearing intraocular ovarian transplants, show normal vaginal cycles and repeated development of vesicular follicles and white corpora lutea(1). Similar hosts, with intraocular pituitary transplants in addition, exhibit pseudopregnancy cycles (2), as observed in intact mice with pituitary isotransplants(3). These cycles are correlated with the development of red corpora lutea in the ovarian transplants. The corpora, unlike those in hosts without pituitary transplants, are functional.

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In the present study, ovarian transplants were placed intraocularly in lactating mice which were simultaneously ovariectomized. Transplants were observed throughout the lactation period for evidence of the action of follicle stimulating (FSH), luteinizing (LH) and luteotrophic (LTH) hormone; an attempt was made to prolong the period by substituting suckling young with newborn mice when the former were 2 weeks of age.

Material and methods. Twenty BALB/c mice, each of which had delivered a litter within the same 48 hour period, were matched with 20 virgin females of the same

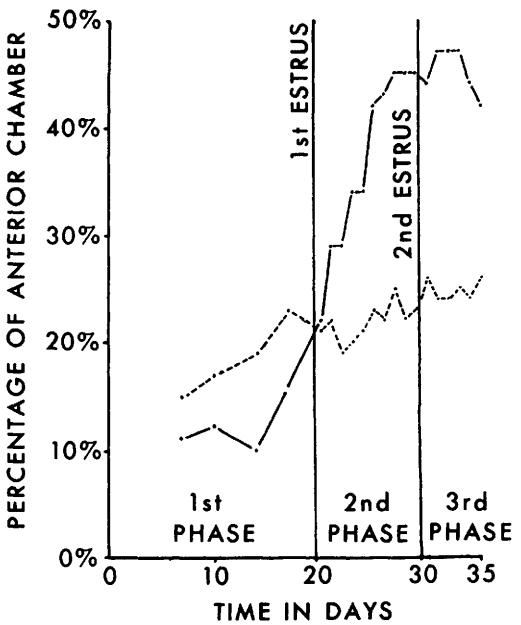


FIG. 1. Percentage area of anterior chamber occupied by ovarian transplants in lactating (———) and non-lactating (– – – –) ovariectomized mice (18 transplants/group) with mean time of appearance of the first and second estrus in lactating mice.

age and strain. The lactating females were castrated within 24 hours of parturition, and each immediately received an isotransplant of ⅛ of an adult ovary, from a donor of the same age, in the anterior chamber of each eye. The virgin females were treated similarly, each within 10 minutes of the corresponding lactating female.

Transplants were observed from the end of the 1st week, and vaginal smears were taken daily (by lavage) from time of transplantation, until the 35th day when experiment was terminated. Lactating mice with litters of less than four were rejected. Litters of the remaining mice were replaced at the end of the 2nd and 4th weeks with the original number of newborn mice (4 to 8). Representative females were sacrificed at intervals and their intraocular grafts removed for histological purposes. Nine pairs of animals completed the experimental period.

Results. In virgin females, intraocular grafts had become vascularized and were growing by the end of the 1st week. The average amount of anterior chamber occupied, in surface area, was 15% on the 7th day, 23% by the 17th day, and only 26% at termination of experiment (Fig. 1).

By the 10th day, all transplants showed vesicular follicles and, by the 14th to 17th days, corpora lutea; thereafter cyclic development of the two occurred. Vaginal smears sometimes showed uninterrupted estrous cycles after ovariectomy and transplantation but often exhibited a prolonged diestrus and erratic cycles, especially in the first 20 days. The corpora lutea, newly formed after estrus, occasionally had a pink color. Such corpora often occurred in only one anterior chamber, those in the opposite eye having the usual opaque white appearance. The pink corpora lasted usually only one day, with immediate reversion to the white type (Fig. 2). Mean length of 10 estrous cycles occurring after the 20th day was 7.7 days, with an associated diestrous interval of 3 days.

In lactating females, growth and development exhibited 3 phases. The *first* occupied the initial 17 to 21 days after ovariectomy and transplantation. During this period, transplants resembled those in virgin females in vascularity and development of vesicular follicles, but the latter did not luteinize. Transplants occupied 11% of the anterior chambers at end of the 1st week and showed no growth by the end of the 2nd week; however, by the 17th day, they occupied 16% and, by the 20th day, 22%, having reached the size of those in virgin females.

Between the 17th and 21st days, the first estrus occurred, marking the beginning of the *second phase.* At estrus (or, occasionally, at proestrus or the day after estrus), the vesicular follicles of all transplants abruptly transformed into corpora lutea with a distinct pink or an intense red color (Fig. 2). Anastomosing vessels on the graft surfaces became engorged. All pink corpora became red after 1 to 3 days; red corpora persisted for 2 to 6 days. They then either reverted to the pink type for 1 to 3 days or, more usually, lost all coloration as abruptly as they had gained it, and resembled white corpora lutea in virgin females. The total

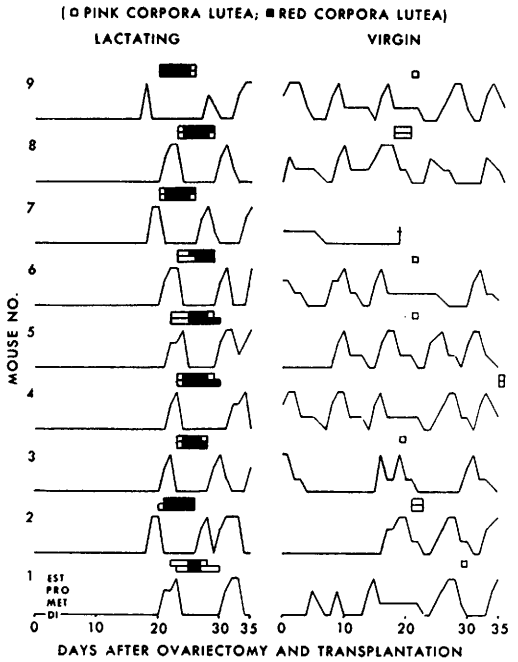


FIG. 2. Vaginal estrous cycles and time of appearance of pink or red corpora lutea in intraocular ovarian transplants in ovariectomized lactating or virgin mice.

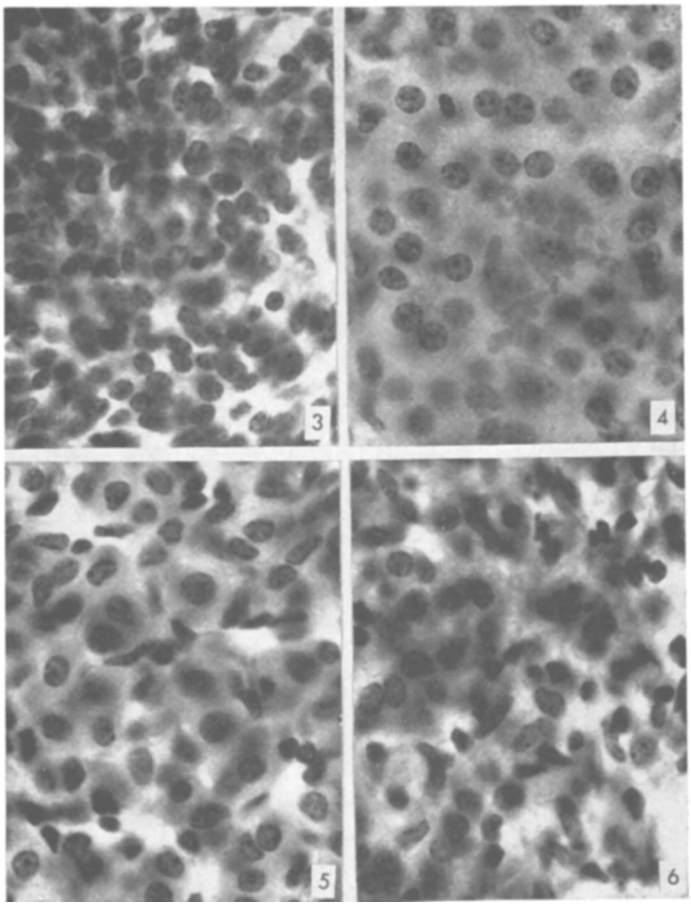
period during which corpora were pink or red was remarkably constant, being 6 days for 12 of the 18 transplants, 5 or 7 days for 5, and 8 days for the remaining one. During this second phase, vaginal smears were of the diestrous type, with a mean duration of 6.5 days and a range of 5 to 9 days. Concurrently with this phase, the surface area occupied by the intraocular grafts increased from 22% to 45% (Fig. 1).

On the 1st or 2nd day after reversion of the corpora to the white type (Fig. 2), a new estrous cycle commenced (except in one animal where it began on the last day of red corpora), marking the beginning of the *third phase*. The mean interval between vaginal estrus at the beginning of the second and third phases was 8.9 days. The corpora lutea regressed, slowly losing their distinct outlines, and were replaced by vesicular follicles and new corpora. The latter occasionally showed a pink color for one or 2 days. During this third phase, the females became unable to maintain their substituted litters. New estrous cycles began to follow without development of red corpora lutea or a prolonged diestrus, ending the third phase. Transplants showed no further growth in this period.

Histologically, pink corpora lutea were composed of closely-packed cells with large nuclei and scanty cytoplasm (Fig. 3). They often showed mitotic activity, accompanied by an increase in amount of cytoplasm. Red corpora lutea consisted of cells with comparatively large amounts of cytoplasm, very uniform nuclei, and islands of red blood cells (Fig 4). After reversion to white corpora in the early part of the third phase, the cells still retained relatively abundant cytoplasm, but cell boundaries were unusually distinct, nuclei less uniform, and islands of red blood cells rare or absent (Fig. 5). As these corpora lutea regressed in the third phase, the cytoplasm diminished in amount and nuclei became irregular. Corpora lutea of virgin females resembled the pink corpora of lactating females, except for greater variability in nuclear size and extensive vacuolization of the cytoplasm (Fig. 6).

Discussion. The development of vesicular follicles in intraocular ovarian isotransplants in ovariectomized mice, in contrast to the minute immature follicles seen in such transplants in intact mice, indicates the action of pituitary FSH; luteinization, which follows vaginal estrus in the ovariectomized animals, indicates pituitary release of LH(1,4). Uterine deciduomata cannot be induced in similar animals, bearing pituitary as well as ovarian transplants, unless *red* corpora lutea are present. The red color, presumably due to sinusoidal engorgement, indicates LTH release by the pituitary transplant, with consequent progesterone secretion by the red corpora (2).

Inferentially, in the present study, pituitary release of FSH continued in lactating mice, for vesicular follicles developed in their intraocular ovarian transplants. However, in comparison with virgin mice, this release may have been at a low level, for appreciable growth was absent until late in the lactation interval. On the other hand, LH release did not occur, for the follicles did not luteinize as



Intraocular ovarian isografts in lactating or virgin female mice. ×665.

FIG. 3. Pink corpus luteum showing closely packed nuclei, indistinct cell boundaries and scanty cytoplasm; early *second phase*, lactating female.

FIG. 4. Red corpus luteum showing uniform nuclei, abundant granular cytoplasm, and areas of red blood cells; *second phase*, lactating female.

FIG. 5. White corpus luteum, showing irregular nuclei, distinct cell boundaries and absence of red blood cells; early *third phase*, lactating female.

FIG. 6. White corpus luteum, showing very variable nuclear size and extensive cytoplasmic vacuolization; *cyclic* virgin female.

they did in virgins. Presumably, LTH was being secreted continuously in relation to lactation but, in the absence of luteinized follicles, its luteotropic effects could not be expressed. As the end of the lactation interval approached, LH release occurred, for corpora lutea developed and were immediately converted into the functional (red) type by the pre-existing LTH. At the same time, FSH release was also stepped up for transplants showed very active growth in this period (end of 1st phase).

Rothchild(5,6) considered that both FSH and LH were virtually absent during lactation, and pseudopregnancy, owing to suppression of their formation in the pituitary by the suckling stimulus in the former and to inhibition of their release from the pituitary by progesterone in the latter. The present findings are compatible with this hypothesis although LH rather than both FSH and LH secretion was suppressed during the lactation interval (1st phase). In the subsequent pseudopregnancy (2nd phase) functional corpora lutea were present and their disappearance was followed by a new estrus. However,

exogenous progesterone(4) gave no evidence of inhibition of FSH or LH release in castrated female mice bearing ovarian transplants (although it did produce complete inhibition of such release in intact males with similar transplants). In any case, as has been suggested(7), the lactation interval is not under luteal control for no corpora lutea were present during the 1st phase.

Despite maintenance of a strong suckling stimulus by litter substitution, lactation began to fail after the end of the 1st month (beginning of the third phase), although lactation has been prolonged to 2 months in intact mice by the same technic(8). While the amount of ovarian tissue in bilateral intraocular transplants is considerably less than in the ovaries *in situ*, it is sufficient to reestablish estrous cycles in virgin mice. Further, only LTH and corticosteriods are essential for lactation(9) over short intervals at least; possibly prolonged lactation requires a higher level, or a different type, of ovarian hormone than the small and ectopic intraocular tissue can supply.

The occurrence of estrus in the 3rd week after parturition, ending a more or less constant "lactation interval," but with continued lactation through one or more "pseudopregnancy cycles," has been observed before (5. 8). The lactation interval evidently corresponds to the *first phase* of the present study; the *second phase*, with red corpora lutea, is the equivalent of a pseudopregnancy cycle. Such an event, with functional corpora lutea like those formed after the immediate postparturitional estrus of the intact mouse, and accompanied by a far longer period of uterine decidual reactivity than in gestation(10), seems to be coincidental with, rather than essential for, lactation.

Summary. 1. Intraocular ovarian isotransplants in ovariectomized lactating BALB/c mice showed little growth and developed only vesicular follicles in the first part of the lactation interval. The first vaginal estrus occurred between 17 and 21 days after parturition, followed by abrupt appearance of pink or red corpora lutea. 2. These atypical corpora persisted from 5 to 8 days, concurrently with vaginal diestrus. They then became white and a second vaginal estrus occurred, unaccompanied by red corpora. Transplants showed considerable growth between the first and second estrus. 3. Similar transplants in ovariectomized virgin females developed vesicular follicles and corpora lutea from the 10th day onwards, with more or less normal estrus cycles. Corpora were typically white. Growth of transplants was moderate but continuous. 4. It is suggested that red corpora lutea indicate the action of LTH; and that pituitary release of LH, but not of FSH, is inhibited over most of the lactation interval.

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