THE MAINTENANCE OF GESTATION AND THE INITIATION OF LACTATION IN THE MOUSE IN THE ABSENCE OF PITUITARY PROLACTIN

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Summary. Dwarf mice with hereditary prolactin deficiency were rendered fertile by implanting normal mouse pituitary under the kidney capsule. The grafts were removed by unilateral nephrectomy between Days 9 and 16 of pregnancy. All females carried to term and delivered litters. The young died within 2 to 8 days, apparently from lack of milk. The comparison of these results with the effects of hypophysectomy during pregnancy suggests that the mouse placenta produces prolactin-like hormone before it can provide full luteotrophic complex. The involution of mammary glands is slower in dwarfs deprived of the source of prolactin but secreting LH, FSH and ACTH than in the hypophysectomized animals.

Mice hypophysectomized during the second half of pregnancy will carry to term (Seyle, Collip & Thomson, 1933) and may deliver live young (Newton & Richardson, 1941) but the gestation may be somewhat prolonged and the delivery is often difficult or incomplete (Gardner & Allen, 1942). The maintenance of functional corpora lutea in hypophysectomized pregnant mice depends on the presence of placenta (Deanesly & Newton, 1941) which secrete 'mammatrophic' substance (Kohmoto & Bern, 1970). The placenta apparently also produces an FSH-like hormone which, together with prolactin, constitutes a 'luteotropic complex' in the mouse (Choudary & Greenwald, 1969b). In hypophysectomized pregnant mice, mammary glands seem to develop normally but regress soon after parturition and all young die within 36 hr of birth (Newton & Richardson, 1941). Similarly, in mice hypophysectomized during lactation, the milk production ceased within 24 hr (Selye *et al.*, 1933).

In the present study, the maintenance of gestation and the initiation of lactation were observed in mice deprived of pituitary prolactin at different stages of pregnancy but still secreting most of the other adeno-hypophysial hormones. This was achieved by using genetically dwarf mice which have a hereditary prolactin deficiency (Bartke, 1967; Cheever, Seavey & Lewis, 1969) but secrete LH and FSH and can reproduce when treated with prolactin (Bartke, 1965, 1966).

Twenty-four dwarf (dw/dw) females derived from crossing Dw/Wf, YS/ChWf-dw and YS/ChWf strains were used. They were from 3 to 14 months old and weighed from 8.0 to 18.5 g (mean 14.2 g). Since in these animals puberty does not occur spontaneously, they were treated with 3.0 μ g DL thyroxine Na intraperitoneally three times a week for a month and 4.5 μ g DL thyroxine Na intraperitoneally three times a week for another 4 weeks. This resulted in some growth (average weight gain, 3 g) and establishment of vaginal opening. Forty-three days after the onset of treatment, each dwarf mouse received a homograft of one pituitary from a normal adult female under the right kidney capsule to provide a source of prolactin (Everett, 1964). The dwarfs were mated to normal adult males 10 days later and checked daily for the presence of vaginal plugs. Of twenty-four animals, twenty-two mated and fifteen became pregnant. Maternal behaviour and lactation were normal and, of thirty-four young born to females used in this study (Table 1), thirty-one survived to weaning and subsequently developed into apparently normal fertile animals.

Female dwarf mice rendered fertile by a pituitary homograft were then used to study the effects of the absence of pituitary prolactin on the maintenance of pregnancy and the initiation of lactation. Seven animals of proven fertility (one or two litters) and two other visibly pregnant females were unilaterally nephrectomized between Days 9 and 16 of gestation (counting as Day 1 the day on which the plug was found) to remove the pituitary homograft. There was no mortality and the general condition of the animals seemed perfectly normal.

All animals deprived of the source of pituitary prolactin (i.e., nephrectomized) during pregnancy carried to term and seven delivered live young (Table 1). The parturition was apparently normal; the length of gestation and the litter size were not affected. The mean litter size with and without the removal of pituitary graft was 4.8 ± 0.5 and 4.8 ± 0.8 , respectively. The mean number of live young per litter was, correspondingly, 3.2 ± 0.8 and 3.4 ± 0.9 . The maternal behaviour was apparently normal, the young were actively suckling and some milk seemed present in their stomachs during the first few days after birth. However, they failed to grow normally, looked progressively more and more emaciated and none survived longer than 8 days.

After the litters died, females were mated to males of known fertility and checked daily for vaginal plugs. The plugs were observed at regular 4- or 5-day intervals in all animals. The absence of pregnancy or pseudopregnancy after fertile mating showed that the animals were indeed prolactin deficient, i.e. the removal of the graft was complete. Dwarf females bearing pituitary grafts are usually capable of producing a series of litters (Table 1 and Bartke, 1965).

These results demonstrate that, in the mouse, pituitary prolactin is not essential for the maintenance of pregnancy after Day 9, for normal delivery and maternal behaviour or for the mammary development and initiation of lactation, but is essential for the maintenance of milk production.

Pregnancy was maintained when the source of pituitary prolactin was removed at Day 9 or 10, the time when hypophysectomy will interrupt pregnancy (Choudary & Greenwald, 1969a). It should be noted that Days 9 and 10 in the present study probably correspond to a less advanced stage of foetal and placental development than that in mice used by Choudary & Greenwald

TABLE 1
EFFECTS OF REMOVING PITUITARY HOMOGRAFTS ON PREGNANCY AND LACTATION IN DWARF MICE

<i>No. of litters</i>	<i>Fertility before graft removal Litter size</i>	<i>Day of graft removal (plug day = Day 1)</i>	<i>Outcome of pregnancy</i>	<i>Length of the survival of the young</i>
2	7 (1 dead), 5	9	4 live young	4 days
2	7, 4 (3 dead)	9	Stillborn or cannibalized litter	—
1	? (stillborn)	10	1 live and 4 dead young	2 days
2	1, 5	11	5 live young	3 days
—	—	12	5 live young	6 days
—	—	13	5 stillborn young	—
1	? (stillborn or cannibalized)	15	2 live young	2 days
1	7	15	7 live young	4 days
1	2	16	5 live young	8 days

(1969a) since the length of pregnancy was 19 to 21 days in the dwarfs (both before and after nephrectomy) and 18 to 19 days in their animals. The maintenance of pregnancy in mice deprived of the source of pituitary prolactin on Day 9 or 10 of gestation seems to imply that the mouse placenta produces prolactin-like hormone at least 2 days before it is capable of secreting complete 'luteotropic complex'. This interpretation is supported by the findings of Kohmoto & Bern (1970) who demonstrated mammotrophic activity of mouse placental tissue on Day 6 of pregnancy and suggested that this placental lactogen could enter the circulation from Day 8 (in the present study, this corresponds to Days 7 and 9, respectively).

The involution of mammary glands after parturition was much slower in nephrectomized dwarfs than in the hypophysectomized animals. Similarly, slow inhibition of milk production was previously observed in dwarf females which had pituitary grafts removed during lactation (Bartke, 1965).

A number of differences between the hereditary dwarf and the hypophysectomized mice could have contributed to their different reproductive performance after the removal of the pituitary homograft and the hypophysectomy, respectively. Dwarf mice lack prolactin and growth hormone and are hypothyroid (Bartke, 1967; Lewis, 1967) but secrete LH and FSH in apparently normal manner (Bartke, 1965), have an unaffected neurohypophysis (Peterson, 1959) and produce significant, if not normal, amounts of ACTH (Shire, personal communication). They seem uniquely suitable for the study of the functions of pituitary and placental prolactin.

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