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# Some effects of mastectomy on reproductive success in the guinea-pig

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**Summary.** Virgin guinea-pigs were mastectomized in two stages between 11 and 18 weeks of age and then mated, starting 19 weeks after final surgery. In the subsequent first pregnancy, the incidence of still-births and neonatal deaths was significantly higher in the mastectomized animals (6 out of 12 mothers (50%) and 14 out of 49 young (29%) compared with intact guinea-pigs (1 out of 15 mothers (7%) and 1 out of 58 young (2%)). There was no significant effect of mastectomy on litter size and weight or on gestation period. The still-born were not significantly different in weight from those born alive. A significant relation was found between maternal weight changes in the period 20 to 5 days before parturition and the occurrence of still-births and neonatal deaths; still-births were associated with a period of reduced weight gain. No effect of mastectomy on the length of the oestrous cycle was apparent but a significant increase in the incidence of non-pregnancy was found. The results provide further evidence that mastectomy influences reproductive success in the guinea-pig and suggest that parturition is a key process affected.

*Keywords:* mammary gland; parturition; still-birth; pregnancy; guinea-pig

## Introduction

In 1980, Peaker & Maule Walker reported that mastectomy decreased the length of the oestrous cycle in a group of goats; later, effects on parturition in these same animals became manifest (Maule Walker & Peaker, 1981). These findings questioned the traditional view that the mammary gland receives many hormonal signals but sends none, and a new concept developed in which the mammary gland is viewed as having a role in the integration of reproductive processes (see Peaker, 1982; Diamond, 1982).

Because of the long period required to follow up these observations, another species was sought in which mastectomy is a relatively simple and humane procedure and in which tests of hypotheses on possible mechanisms could be made. In addition, of course, such studies would provide information on whether the phenomenon occurs in other species. While the guinea-pig, with two inguinal mammary glands, appeared ideal, previous studies in mastectomized animals had provided either some (Marshall & Kirkness, 1907) or no (Linzell, 1963) indication of an effect of mastectomy on reproduction (see 'Discussion').

In a preliminary series from this laboratory, Calvert & Peaker (1986) found that when young guinea-pigs were totally mastectomized they died at the end of their first pregnancy but that when some mammary tissue had been left *in situ*, parturition occurred normally. We now report studies on another group of mastectomized guinea-pigs.

## Materials and Methods

Guinea-pigs (Dunkin–Hartley) were kept in a 12 h light:12 h dark photoperiod; they had guinea-pig breeder and stock diet (Labsure, Manea, Cambs, UK) and drinking water supplemented with ascorbic acid available at all times. They

were mastectomized in two stages: one mammary fat pad together with the teat was removed at 11–12 weeks of age, the other at 17–18 weeks. Animals were anaesthetized each time with ketamine/xylazine as described by Suckling (1987); recovery from anaesthesia was rapid and uneventful, and the animals were behaving normally within several hours. In view of the earlier finding that reproduction in incompletely mastectomized guinea-pigs was unaffected by the operation, i.e. the animals had undergone exactly the same procedure but some mammary tissue had inadvertently been left *in situ* (Calvert & Peaker, 1986), the mastectomized animals were compared with intact guinea-pigs of the same age. In virgin guinea-pigs the limits of the mammary fat pads are indistinct and mammary tissue can develop during pregnancy from remnants left *in situ*. Because we have previously found that total removal of mammary tissue by excision of the mammary fat pad in young animals is difficult to achieve, the presence of residual or regenerated mammary tissue was determined at autopsy 2 days *post partum*.

Starting at 19 weeks after the second gland was removed, males were introduced into the cages on the first day of vaginal opening and removed after closure; this procedure was repeated at each cycle until the animals became pregnant (1–3 cycles in the mastectomized and intact control animals, although 2 of the 14 mastectomized animals did not become pregnant after 7 cycles and were excluded from further study). The first successful matings were 20 weeks after final surgery. The day of maximal vaginal opening was defined as Day 1 of the oestrous cycle or of the subsequent pregnancy. Animals were monitored to study the lengths of the oestrous cycle and gestation, weight changes during pregnancy and survival of mothers and young. Still-births were taken as those young found dead in the cage immediately after parturition (the still-born were found surrounded by fetal membranes and with unexpanded lungs) and neonatal deaths as young found dead later but within 8 h of parturition.

## Results

### Completeness of mastectomy

In 6 of the 12 mastectomized animals, small amounts of milk-filled mammary tissue were found (in 2 the amount was too small to be dissected out to weigh; in another 2 the weight was 0·5 g and in the other 2 it was ~ 1 g). No mammary tissue was found in the other 6 animals. From the weight of the mammary glands in intact guinea-pigs in early lactation (mean 2·7% of bodyweight, see data collected by Linzell, 1972) and the weight of the mastectomized animals, it was calculated that, overall, the weight of mammary tissue in the mastectomized animals had been reduced to $<4\%$ of that in intact animals.

### Oestrous cycle

No effect of mastectomy on the length of the cycle was apparent (16·5 $\pm$ 0·34 (s.e.) days in 14 intact animals over 18 cycles: 16·8 $\pm$ 0·23 days in 14 mastectomized animals over 38 cycles), as found previously (Calvert & Peaker, 1986).

### Incidence of pregnancy

As noted in the 'Materials and Methods', 2 of the 14 mastectomized animals did not become pregnant after 7 cycles. During the period of study, 27 intact animals were mated for other experiments; all became pregnant after 3 or fewer cycles. Using these data, the incidence of non-pregnancy was significantly higher in the mastectomized animals ($P < 0{\cdot}05$, $\chi^2$ test).

### Pregnancy and parturition

Of the 12 mastectomized mothers, 5 had part of the litter still-born compared with 1 out of 15 in the controls ($P < 0{\cdot}02$, $\chi^2$ test); similarly, 9 out of 49 young were still-born to mastectomized mothers compared with 1 out of 58 to the controls ($P < 0{\cdot}005$, $\chi^2$ test). There were no neonatal deaths in the intact controls and 5 in 3 mastectomized mothers; 2 of these 3 mastectomized animals also had still-born young. Therefore, 6 out of 12 mastectomized guinea-pigs had still-births and/or neonatal deaths ($P < 0{\cdot}02$, $\chi^2$ test) and 14 out of 49 young born to the mastectomized animals were still-born or died shortly after birth ($P < 0{\cdot}001$, $\chi^2$ test). There was no apparent relation between the occurrence of still-births plus neonatal deaths and completeness of mastectomy.

In guinea-pigs, the incidence of still-births and neonatal deaths has been found to increase with litter size (Haines, 1931; Goy *et al.*, 1957). However, there was no significant effect of mastectomy on litter size at delivery (3·6 ± 0·20 in 16 intact; 4·0 ± 0·17 in 12 mastectomized, median 4 in both) or on total weight of young (including still-born) (360 ± 23·5 g and 397 ± 25·3 g, respectively). The incidence of still-births has also been found to be higher with gestation periods longer and shorter than 69 days (Goy *et al.*, 1957), but there was no significant effect of mastectomy on the length of pregnancy: the gestation period in 8 intact animals in which the time of mating was known was 68·8 ± 0·49 (s.e.) days compared with 67·6 ± 0·43 days in 12 mastectomized (median 68 days in both). The effects of mastectomy on the incidence of still-births could not therefore be attributed to changes in litter size or duration of pregnancy.

There was no indication that the still-born young were larger or smaller than those born alive. Within the litters born to mastectomized animals there was no significant difference (paired *t* test) between the mean weight of the still-born (92 ± 10·4 g) and live (92 ± 6·7 g) young (in the litters with all live young the mean weight was 93·1 ± 4·44 g).

On a subjective assessment it was noticed that some of the mastectomized animals appeared lethargic and weak after parturition and had an unthrifty appearance with a staring coat; as in Calvert & Peaker's (1986) animals, oedema was apparent at autopsy. No indications of infection were present in mothers or still-born young. Such poor condition was not noted before parturition or at any stage in any of the intact animals.

### Changes in maternal bodyweight

In 5 of the 6 mastectomized mothers of the litters in which there were still-births and/or neonatal deaths, the rate of increase in bodyweight during pregnancy appeared to be lower than in those which delivered all live young. In 3 animals, a decrease in the rate of weight gain or a decrease in bodyweight was noted after approximately 48 days of pregnancy; in another 2 after approximately 56 days. In all but 1 of these animals the rate of weight gain increased again nearer term. Within these mastectomized animals, therefore, there were no significant differences in weight between those mothers with still-births and/or neonatal deaths and those delivering all young alive on the day before parturition (1381 ± 39·8 g and 1390 ± 45·5 g, respectively) or on the first day *post partum* (933 ± 34·2 g and 972 ± 19·0 g).

Taking a weight gain of $>25$ g or $<25$ g in any period of 7 days from Day 20 *pre partum* to Day 5 *pre partum* as an arbitrary classification, 6 animals had weight changes of $<25$ g and, of these, 5 had still-births or neonatal deaths. Of the remaining 6 animals, which had a weight gain of $>25$ g per week throughout the period, only 1 had still-births. There was, therefore, a significant relation between a period of low maternal weight gain during the period of 20–5 days before parturition and the incidence of still-births and neonatal deaths ($P < 0·02$, $\chi^2$ test).

## Discussion

The present studies provide further evidence that reproductive disturbances may supervene after mastectomy in the guinea-pig. The results indicate a dysfunction of parturition since the still-born young of the mastectomized animals were fully developed and not significantly different in weight from those born alive. There was no evidence to suggest that mastectomy affected litter size or weight or the length of gestation. There were further, but subjective, indications of dystocia: the mastectomized mothers were, in general, weak after birth and the cages had blood-stained bedding. Maule Walker & Peaker (1981) noted that failure of the uterine cervix to dilate was a key feature in mastectomized goats. The present findings in the guinea-pig, as well as those of Calvert & Peaker (1986) in which there were maternal deaths without delivery of young, could be interpreted in a similar manner.

Although the evidence points to an effect of mastectomy at parturition, an influence on events earlier in pregnancy cannot be excluded. A transient period of low weight gain during late pregnancy in the present studies was significantly related to the occurrence of still-births and/or neonatal deaths; in the mastectomized goats of Maule Walker & Peaker (1981) temporary peripheral oedema was noted in pregnancy.

There was also an indication of an increased incidence of infertility in the mastectomized animals. Similar indications are evident in the data of Marshall & Kirkness (1907) for the guinea-pig and of Peaker & Maule Walker (1980) for the goat.

The results of earlier studies of mastectomy in the guinea-pig are variable. Linzell (1963), who mastectomized guinea-pigs to follow up his finding of an apparently higher rate of reproductive disturbances in goats which had lost large amounts of mammary tissue during early attempts at autotransplantation, found no deleterious effects on the mother; the incidence of still-births and neonatal deaths was not reported. Lebedeva & Taranenko (1978) also reported no effect but found that extensive regeneration of mammary tissue had occurred. By contrast, Calvert & Peaker (1986) found that 5 out of 11 guinea-pigs mastectomized before mating died in late pregnancy; another produced 4 dead and 1 live young but both mother and young died shortly after. In all 6 animals that died no trace of mammary tissue was found but in the other 5, all of which delivered live young, relatively large amounts of mammary tissue were found *post partum*. While the development and regeneration of mammary tissue may be an important source of variation, differences in strain cannot be ignored. Goy *et al.* (1957) found marked differences in the incidence of still-births between different strains of guinea-pig. The incidence in the present series (1 out of 15 or 7% in the intact controls), albeit in a small number of animals, was much lower than the lowest reported by Goy *et al.* (1957) but similar to that (5%) found by Rowlands (1949). The effects of mastectomy in guinea-pigs of strains with a higher incidence of still-births would be interesting.

The question of the mechanism that might be responsible for the effects of mastectomy remains. Since the response to mastectomy appears to be variable, a modulatory mechanism rather than an invariable, obligatory control seems most likely. Although lack of completeness of mastectomy and variation in the stage of life at which mastectomy was performed are complicating factors, and genetic (strain) differences cannot be ignored in considering earlier studies, there do appear to be differences in the severity of response in different studies on the same species; an obvious example is the difference between the present series of experiments and that of Calvert & Peaker (1986). There is no indication that this difference is related to the length of the period between surgery and parturition or to the condition of the animals; in both studies the weight of the mastectomized animals was not significantly different at the time of mating. Furthermore, in the goat, significant effects of mastectomy have been evident months and years after surgery in some studies (Peaker & Maule Walker, 1980; Maule Walker & Peaker, 1981; C. H. Knight, E. Taylor & M. Peaker, unpublished) but not in others (Lebedeva & Taranenko, 1978). Similarly, in Linzell's (1963) work, in which data on reproductive disturbances were obtained incidentally to the main purpose, there was clearly great variation between animals. In cattle (Short *et al.*, 1972; Grass & Hauser, 1981) and pigs (Buttle, 1987) the only effect of mastectomy that has been evident is a reduction in the time between parturition and the first oestrus *post partum*.

The mechanisms by which absence of the mammary gland is signalled and by which the effects of mastectomy are exerted are unknown. However, since relaxin is involved in the events of late pregnancy, it is possible that alterations in its rate of synthesis and/or secretion could cause disintegration between the processes of uterine quiescence, uterine contractility and cervical dilatation in which it is involved. Indeed, immunological neutralization of relaxin in the rat during the second half of pregnancy has been shown to result in prolonged parturition and an increased number of still-born young (Lao Guico-Lamm & Sherwood, 1988; Hwang *et al.*, 1989). In the guinea-pig, which delivers much larger fetuses than the rat, a much greater degree of cervical dilatation and pelvic relaxation is required (see Porter, 1983). The hypothesis that mastectomy might alter the production of relaxin is now being tested and there is preliminary evidence from immunohisto-

chemical studies that the content and distribution of relaxin in the endometrial glands, the main source of relaxin in the guinea-pig, are different in mastectomized animals during late pregnancy (Peaker *et al.*, 1989a). However, even if one of the effects of mastectomy is on relaxin synthesis or secretion, the mechanism by which the absence of the mammary gland is signalled to the endometrial gland cells and whether this phenomenon is related to the synthesis of relaxin by the mammary gland (Peaker *et al.*, 1989b) remain to be determined.

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