ENDOCRINE ACTIVITY OF THE EARLY PLACENTA OF THE GUINEA-PIG

RUTH DEANESLY
National Institute for Medical Research, Mill Hill, London, N.W. 7

(Received 30 May 1960)

SUMMARY

Mucification of the vaginal epithelium in rodents requires the presence of both oestrogens and progesterone.

The effect of different doses of hormones on the vaginal epithelium of spayed animals is briefly described.

In spayed pregnant guinea-pigs maintained by progesterone, mucification of the vagina similar to that of pregnant controls develops by about days 18–20 post coitum, indicating a secretion of oestrogen by the developing placenta adequate to transform the vaginal epithelium, but probably of < 1 μg daily.

INTRODUCTION

The vaginal epithelium of the guinea-pig undergoes striking changes during the sexual cycle and pregnancy. They have been studied in detail by Courrier and his collaborators (Courrier, 1925, 1945) and by others including Tribby (1943). Like the mouse, rat and hamster, the guinea-pig shows vaginal mucification in pregnancy. Experimental work in the rat and hamster (Klein, 1935, 1937; Selye, Browne & Collip, 1936) has proved that similar mucification can be produced by oestrogen and progesterone acting together but not by either alone, unless a relatively enormous dose of progesterone is given (Selye, 1940).

Herrick (1928) first showed that pregnancy could continue in the guinea-pig after ovariectomy carried out on, though not before, day 27. Courrier, Kehl & Raynaud (1929) confirmed these results and stated that pregnancy might go to term after ovariectomy as early as day 20. They found that vaginal mucification was maintained in ovariectomized pregnant guinea-pigs and moreover, as in intact pregnant controls, there was no vaginal opening in response to oestrogen injections. Courrier et al. concluded that, in the absence of the ovaries, the injected oestrogen was neutralized in the pregnant animals, possibly by the placenta.

Recent experiments (Deanesly, 1960a, b), extending the early work of Loeb & Hesselberg (1917), have shown that implantation will occur normally in guinea-pigs ovariectomized on days 3–5 post coitum (p.c.). Development will continue up to days 11–14 without exogenous hormone and thereafter if 10 mg progesterone is injected daily. This finding gives an opportunity to estimate the first appearance of placental endocrine activity by comparing the vaginal epithelium in pregnant and non-
pregnant ovariectomized guinea-pigs belonging to the same experimental groups (Deanesly, 1960b) and receiving either no extraneous hormones or progesterone alone, up to day 20 p.c.

MATERIAL AND METHODS

The guinea-pigs were from the albino colony maintained at the National Institute for Medical Research. Ovariectomies were carried out as previously described (Deanesly, 1960b). No ligatures were used on either uterine tube or uterus. Progesterone and oestradiol benzoate were injected subcutaneously in oil. Histological material was fixed in Bouin’s fluid and stained in haematoxylin with or without eosin. Part of the material was also stained specifically for mucin with periodic acid-Schiff and Orange G, and I am indebted to Dr M. Allanson for these slides.

I. Vaginal epithelium in normal pregnant females

The vaginal epithelium was examined in seventeen pregnant guinea-pigs up to day 28 p.c. Well developed mucified layers were found in one animal by day 10, whereas in others at day 12 or day 14 the epithelium was lower and less mucified, though with a continuous layer of columnar cells on day 14 (Pl. 1, fig. 3). No further development was shown by one 18-day female with four embryos, whereas in three others, 17–18 days pregnant, including one in which resorption was taking place, the mucified epithelium showed further thickening and infolding, as shown in Pl. 1, fig. 4. At 20–22 days pregnant, three out of four guinea-pigs showed continued growth and development of the mucified epithelium (Pl. 2, fig. 5); in the other the vaginal epithelium was nearer to the 14-day type (Pl. 1, fig. 3). By 28 days there was a marked further increase and thickening of the epithelium (Pl. 2, fig. 7).

Although it shows variations in thickness in the same section as well as between different females in the same stage of pregnancy, the epithelium becomes consistently thicker and more elaborate during the 1st month of pregnancy. This probably indicates an increased placental production of oestrogens (p. 237).

II. Vaginal epithelium in ovariectomized, non-pregnant guinea-pigs

(a) Guinea-pigs receiving no hormone

Six guinea-pigs, mated and unmated, were killed 6–7 days after ovariectomy and one 71 days after ovariectomy. The mated females can be compared with the early pregnant females described in §III(a). In all these the vaginal epithelium is low and of the type shown in Pl. 1, fig. 1. As pointed out by Courrier (1945), the cells next to the lumen may contain traces of mucin, but this single layer of small cells is distinct from the mucification found in pregnancy or after administration of progesterone and oestrogen. In this group the mucified layer was best defined in the guinea-pig killed 71 days after ovariectomy.

(b) Guinea-pigs receiving oestrogen only

Two guinea-pigs received 1 µg oestradiol daily for 7 and 8 days after ovariectomy on day 2 p.c. Implantation did not occur and at 10 and 12 days p.c. the vaginal epithelium was stratified in both females.
(c) Guinea-pigs receiving progesterone

Six females ovariectomized 3–5 days after mating received 10 mg progesterone daily from days 11 to 20 p.c., but showed no implantations at autopsy on day 21 p.c. In these animals the vaginal epithelium was transitional, low or with a single layer of mucified cells (Pl. 1, fig. 1).

(d) Guinea-pigs receiving progesterone and oestrogen

A female ovariectomized on day 5 p.c. received 5 mg progesterone daily plus 1 µg oestradiol benzoate from days 11 to 20 p.c. There were no implantations at autopsy on day 21, but the vaginal epithelium was deep and mucified, either resting on some stratified layers as in Pl. 1, fig. 2, or mucified right to the basement membrane as in pregnancy (Pl. 2, fig. 5). No epithelium of this type was seen in the previous group which received progesterone only, and it must be concluded that, as in other species, both sex hormones are necessary for this type of mucification.

Four unmated spayed females all received 1 µg oestradiol with 5, 2 or 1 mg progesterone daily for 9 days and were killed 1 day later. The vagina was mucified in all these, but in a similar spayed guinea-pig which received only 0.5 mg progesterone with 0.5 µg oestradiol daily for the same time the mucified epithelium was less developed and contained abundant leucocytes. If the same dose was continued for 12 days and then halved for the following 7 days the epithelium became deeply mucified.

Three other spayed guinea-pigs each received 1 mg progesterone with 0.5 µg, 1 and 5 µg oestradiol benzoate, respectively, daily for 7 days. As in a similar experiment on the rat (Seegar Jones & Astwood, 1942), mucification was best developed in the animal receiving the most oestrogen.

Two experimental spayed females received 1 mg progesterone plus 1 µg oestradiol daily for 8 days and then oestradiol only for the following 7 and 14 days. At autopsy the vaginal epithelium was still deeply mucified in the first but cornified in the second.

III. Vaginal epithelium in ovariectomized pregnant females

(a) Guinea-pigs receiving no hormone

These sixteen animals include some in which resorption of the early embryos was taking place. Up to about day 14 the vaginal epithelium is transitional or low. The inner layer of cells may be very slightly mucified as in non-pregnant ovariectomized females. There is no striking difference in the vaginal epithelium between these eight ovariectomized pregnant females and the controls at the same stages of pregnancy, except that the 14-day control (Pl. 1, fig. 3) shows a more definite single layer of columnar mucified cells. Five ovariectomized pregnant females also had transitional vaginal epithelium at 15–16 days p.c., but one of the three guinea-pigs killed at 18 days showed definite mucification (Pl. 1, fig. 4) not unlike that in corresponding pregnant controls. As already mentioned the extent of vaginal mucification is normally rather variable between days 16 and 21.

One pregnant female was ovariectomized on day 20 and killed 8 days later, having received no hormones. It contained four normal embryos and the vaginal epithelium was deeply mucified and thrown into crypts as in a normal 28 days pregnancy.
The placental secretions had been adequate both for maintenance of pregnancy and of the vaginal mucification.

(b) Guinea-pigs receiving progesterone only

Eight pregnant ovariectomized guinea-pigs received progesterone from day 11 p.c. till 1 day before they were killed at 20 or 21 days p.c., the majority containing living embryos. The vaginal epithelium showed early mucification in all these and contrasted with the vaginal epithelium of similar females receiving the same amounts of progesterone, but showing no implantations (§II c). Pl. 2, fig. 6, shows the well-developed mucification in one of those 20 days’ pregnant guinea-pigs resembling that of a similar intact pregnant control. Two other pregnant females, killed on days 20 or 21 which had progesterone only from day 14, also had a mucified epithelium.

(c) Guinea-pigs receiving progesterone and oestradiol

The vaginal epithelium was examined in three 21 days’ pregnant guinea-pigs ovariectomized on days 3, 4 or 5 and given 5 mg progesterone and 1 µg oestradiol from day 11 to day 20. Mucification was well developed, but not more so than in a non-pregnant, similarly treated animal (Pl. 1, fig. 2).

DISCUSSION

These results show that in ovariectomized guinea-pigs, untreated or receiving progesterone, only traces of mucification occur in the vaginal epithelium. In corresponding ovariectomized pregnant animals, however, at 18–21 days after mating the epithelium is multi-layered and mucified as in control females at the same stage of pregnancy. At about this time the developing placenta apparently produces enough oestrogen to interact with the injected progesterone and to cause the characteristic changes in the vagina. The well-developed mucified epithelium in an ovariectomized non-pregnant guinea-pig receiving 5 mg progesterone and 1 µg oestradiol in addition daily (Pl. 1, fig. 2) shows that 1 µg oestradiol is adequate to convert the low epithelium in such guinea-pigs receiving progesterone (Pl. 1, fig. 1) to the fully mucified type deeper than in the 3rd week of normal pregnancy. Canivenc & Mayer (1951) in rather different experiments on ovariectomized pregnant rats also report that 1 µg oestrogen will cause vaginal mucification where previously absent.

The problem of the earliest stage at which placental tissue shows endocrine activity is an important and interesting one. Chambon (1949) and Chambon & Michon (1952) deduced early oestrogenic activity by the rabbit trophoblast from the fact that implantation could take place in the mated spayed animal if progesterone alone was given, whereas the traumatic decidua in the spayed rabbit required oestrogen as well as progesterone. In the guinea-pig with a 16–17 day normal cycle and implantation at 6–7 days p.c., the placenta’s endocrine (luteotrophic) activity has probably begun by day 15. In the spayed pregnant guinea-pigs the vaginal epithelium differs from that of similar non-pregnant females by day 18–20 p.c., and allowing for some retardation of placental development in the experimental animals the results are not far from the theoretical expectation. Attempts to prolong corpus luteum activity with oestrogens have been unsuccessful and it is probable that the placental luteotrophic hormone is a distinct one.
Endocrine activity of early guinea-pig placenta

With the possible exception of one animal (Pl. 1, fig. 4) only evidence of oestrogenic activity can be deduced up to day 20–21, since extraneous progesterone, not by itself mucifying, must be given to maintain pregnancy in the spayed female.

The absence of vaginal cornification in the ovariectomized pregnant guinea-pig in response to injected oestrogens (Courrier et al. 1929) would seem to indicate a production of progestagens by the placenta. This was confirmed in the present series since mucified vaginal epithelium, once established in the spayed female, broke down after 10 days on 1 µg oestradiol daily in the absence of progesterone.

The fact that implantation and early development can go on in the guinea-pig ovariectomized 3–5 days after mating indicates that the ovarian progestagen remains effective for some time in these animals after spaying. The uninjected ovariectomized guinea-pig showing vaginal mucification at 18 days p.c. (Pl. 1, fig. 4) may, therefore, have derived the necessary minimum of progestin either from the ovariess (removed on day 4) or from the developing placenta.

I am indebted to Miss N. Reid for valuable technical assistance.

REFERENCES


DESCRIPTION OF PLATES

Vaginal epithelium of normal and experimental guinea-pigs. × 198.

PLATE 1

Fig. 1. Low epithelium from a non-pregnant female, 18 days after ovariectomy, which received 10 mg progesterone daily for 10 days before autopsy.

Fig. 2. Mucified epithelium from a non-pregnant female, 16 days after ovariectomy, which received 5 mg progesterone and 1 µg oestradiol daily for 10 days before autopsy. Contrast with Fig. 1.

Fig. 3. Early mucification in a normal 14-day pregnant female.

Fig. 4. Mucified epithelium from an 18-day pregnant female ovariectomized 4 days after mating. This animal received no exogenous hormone.

PLATE 2

Fig. 5. Mucified epithelium in normal 20-day pregnant female.

Fig. 6. Mucified epithelium from a 20-day pregnant female which was ovariectomized at 6 days p.c. and received 10 mg progesterone daily for 9 days before autopsy. Contrast with Fig. 1.

Fig. 7. Further development of mucified epithelium in a normal 28-day pregnant female.

Fig. 8. Well-developed mucified epithelium in a female ovariectomized on day 20 p.c. and killed 8 days later. This pregnant animal received no exogenous hormone, but the vaginal epithelium continued to grow after ovariectomy.