Secretion and reabsorption of uterine luminal fluid in rats

C. A. B. Clemetson, U. L. Verma and S. J. De Carlo

Department of Obstetrics and Gynecology, The Methodist Hospital, 506 Sixth Street, Brooklyn, New York 11215, U.S.A.

Summary. Treatment of ovariectomized rats with oestradiol-17\(\beta\) and progesterone demonstrated that oestradiol-17\(\beta\) causes secretion of sodium, potassium and water into the lumen of the uterine horn and that progesterone causes reabsorption of these substances.

Introduction

Long & Evans (1922) first described the accumulation of fluid which distends the uterine horns of rats at pro-oestrus and oestrus and which is absent at dioestrus. There is no doubt that the horns become distended as a result of oestrogen stimulation; they become empty after ovariectomy and become distended with a fluid similar to that found at oestrus following oestrogen administration (Shih, Kennedy & Huggins, 1940; Meglioli, 1976). Blandau (1945) showed that the cervical canals of the rat are closed by a sphincter-like action at oestrus, but are open at dioestrus, thus permitting escape of the accumulated fluid.

When a high dose of progesterone is given with oestradiol, the accumulation of fluid within the uterine lumen is completely prevented (Armstrong, 1968; Rezabek, 1969, 1972; Tantayaporn, Mallikarjuneswara, De Carlo & Clemetson, 1974). Armstrong (1968) also reported that the fluid disappeared within 12 h after a single s.c. injection of 1 or 2 mg progesterone given 48–66 h after s.c. insertion of an oestradiol implant. However, there is conflicting evidence as to whether this disappearance of luminal fluid after progesterone is due to leakage through the cervical canal or to reabsorption of fluid. Armstrong (1968), using a multiple puncture, blotting and reweighing technique to measure the uterine luminal fluid volume of oestrogen-treated spayed rats, reported ligation of the uterine horns at the cervical ends prevented the fluid loss and therefore concluded that progesterone opens the cervical canals. Meglioli, Krahenbuhl & Desaules (1969) studied, by an aspiration and reweighing technique, oestrogen-treated spayed rats in which the distal ends of the uterine horns had been closed by electrocautery. Progesterone was reported to decrease the volume and increase the viscosity of the uterine luminal fluid even while oestrogen treatment continued, indicating the reabsorption of uterine luminal fluid after progesterone treatment.

Because of these contrary reports, we considered that the question of whether progesterone can cause reabsorption of uterine fluid deserved further study, and the problem was reinvestigated using a different method (uterine washing) for measuring uterine luminal fluid volume.

Materials and Methods

Sexually mature virgin female albino Wistar rats, weighing 200 to 300 g, were given free access to a standard diet (Purina rat chow) and tap water. The animals were bilaterally ovariectomized under barbital anaesthesia 10 days before the start of hormone treatment; part of the oviduct was always removed with each ovary to ensure complete ovariectomy. Silk ligatures (5–0) were placed at the ovarian and cervical ends of one uterine horn of each rat, taking care to avoid injury to the blood vessels.

Ethyl laurate was used as the vehicle for the hormone injections as it had been found to be inert in control studies. At the end of each experiment a lethal dose of sodium pentobarbital was given i.m.
and a blood sample was taken from each rat by cardiac puncture while the animal was still alive. Ligatures were then placed at each end of the non-ligated uterine horns, and both horns were excised and weighed after washing.

Uterine washing was carried out within 10 min of death. A 5% dextrose solution, made up with demineralized water and passed through a resin column to remove any traces of sodium, was injected (2 ml) into the uterine lumen at one end, using a 27-gauge needle and a plastic syringe. The washings were collected from the other end of the horn, using a similar needle and syringe. The volume of the recovered washing fluid, usually 1.9–2.5 ml, was made up to 3 ml with the 5% dextrose. The fluid was transferred to a plastic tube, centrifuged and decanted within 15 min to remove any cells which could exude potassium. The residue was examined for evidence of infection and any rats with leucocytes in the uterine fluid were excluded from the experiment. Also, any specimen which was incomplete because of leakage during collection was discarded. All uterine washings and corresponding glucose blanks were stored in prewashed plastic tubes at 2°C until analysis at the end of the experiment.

All analyses were performed with reference to the same set of standards. All serum samples, glucose blanks and uterine washings were analysed for sodium and potassium by flame photometry (Coleman, No. 51 flame photometer). Glass syringes and sample tubes were not used to avoid sodium leaching.

The uterine luminal fluid volume for each uterine horn was calculated from the results of analysis of each uterine washing by use of a dilution factor, calculated on the assumption that the sum of the molar concentrations of sodium and potassium in the uterine fluid is the same as in serum samples drawn simultaneously. Such [Na+ + K+] isomolarity was first demonstrated by Howard & De Feo (1959) and has since been confirmed by us on all occasions when there has been enough fluid to analyse.

Thus, uterine fluid volume = (uterine washing (UW) volume × UW [Na+ + K+] mmol/litre/ Serum [Na+ + K+] mmol/litre. The uterine fluid electrolyte concentrations were calculated from the uterine washing analyses by use of the same dilution factor.

Text-fig. 1. The mean ± S.E.M. volumes of fluid in the (a) ligated and (b) non-ligated uterine horns of ovariectomized rats at various times after commencement of oestradiol injections (0.2 µg) every 12 h. The points on the dashed lines represent the volumes in the uterine horns of rats receiving 0.5 mg progesterone from 72 h as well as continued oestradiol.
Sixty-four rats were allocated in equal numbers to 8 groups. Group 1 received no hormone treatment; Groups 2, 3, 4, 5 and 6 received an i.m. injection of 0·2 µg oestradiol-17β (Schwarz/Mann Laboratories) every 12 h and were killed at 24, 48, 72, 96 and 120 h, respectively, after the first injection; Groups 7 and 8 received 0·2 µg oestradiol-17β every 12 h and also 0·5 mg progesterone (Schwarz/Mann) (i.m., every 12 h) starting 72 h after the first oestrogen injection, and were killed at 96 and 120 h, respectively. The oestrogen schedule had been previously found to cause and maintain adequate distension of the uterine horns with luminal fluid but not opening of the cervical canals.

**Results**

The rats in Groups 5 and 6 had high volumes of fluid in the ligated and non-ligated uterine horns, but when progesterone was also administered (Groups 7 and 8) the luminal fluid volumes decreased dramatically (Text-fig. 1). Only 1 rat in Group 7 and none in Group 8 showed distension of a ligated horn after progesterone treatment in spite of continued oestrogen administration.

As shown in Table 1, the total sodium and potassium contents of the fluid in the ligated uterine horns were reduced to 0·96% and 1·4% respectively by progesterone treatment (Group 6 versus Group 8).

| Table 1. The effects of hormone treatments (see text) on the mean ± S.E.M. electrolyte content of the uterine luminal fluid of ovariectomized rats (8/group) |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Group | Time killed (h) | K⁺ (mmol/l) | Total K⁺ (mmol/horn) | Na⁺ (mmol/l) | Total Na⁺ (mmol/horn) | K⁺ (mmol/l) | Total K⁺ (mmol/horn) | Na⁺ (mmol/l) | Total Na⁺ (mmol/horn) |
| 1 | 0 | 19·7 ± 2·1 | 834 ± 483·3 | 103·3:2 ± 2·6 | 5128 ± 3079·5 | 21·5 ± 2·5 | 462 ± 286·4 | 101·5 ± 4·3 | 2641 ± 1735·2 |
| 2 | 24 | 28·9 ± 3·7 | 5955 ± 3573·7 | 94·8:6 ± 3·1 | 17,451 ± 10024 | 30·8 ± 2·6 | 832 ± 341·2 | 93·8 ± 3·7 | 2578 ± 938·7 |
| 3 | 48 | 32·7 ± 3·3 | 10,694 ± 3619 | 90·1:5 ± 5·6 | 23,866 ± 6243·4 | 33·0 ± 4·0 | 8799 ± 1689·6 | 89·7 ± 6·1 | 5067 ± 5067·1 |
| 4 | 72 | 31·0 | 7581 ± 3607·8 | 89·6:3 ± 5·6 | 20,083 ± 7068 | 30·3 ± 3·2 | 12,479 ± 3467·7 | 90·3 ± 3·5 | 32,133 ± 8250·5 |
| 5 | 96 | 31·1 | 9108 ± 3476·8 | 96·6:4 ± 5·6 | 29,226 ± 7068 | 32·5 ± 3·2 | 5667 ± 3467·7 | 106·6 ± 3·5 | 26,715 ± 8250·5 |
| 6 | 120 | 19·0 | 6591 ± 3476·8 | 119·3:3 ± 5·6 | 37,056 ± 10,593·7 | 19·9 ± 1·7 | 9858 ± 2367·4 | 118·5 ± 1·4 | 52,077 ± 9566·1 |
| 7 | 120 | 21·4 | 1104 ± 1929·3 | 108·3:7 ± 5·6 | 7395 ± 7457·5 | 24·9 ± 2·6 | 5276 ± 3485·3 | 118·5 ± 7·0 | 25,077 ± 13,798·1 |
| 8 | 120 | 25·5 | 106·4 | 106·4:3 ± 5·6 | 354 ± 27·0 | 126 ± 81 | 100·9 ± 31·8 | 354 ± 6·8 | 105·5 ± 7·2 |

*Significantly different from values for Groups 5 and 6: *P < 0·05; **P < 0·01; ***P < 0·001.
†7 rats only.

Following oestrogen treatment, the potassium concentration in the uterine luminal fluid ([K⁺]ULF) rose significantly at 48 h, but fell again at 120 h. The mean [K⁺]ULF was higher at 120 h in the progesterone- and oestrogen-treated rats, than in those receiving oestrogen alone, but this difference was not significant.

**Discussion**

The effects of oestradiol and progesterone on the volume of fluid in the lumen of the uterus of the rat are quite clear: oestradiol caused the secretion of a fluid, containing sodium and potassium, into the...
lumen of the uterus; a high dose of progesterone, even when given with oestrogen, blocked this secretion and caused reabsorption of water, sodium and potassium from the uterine cavity. Reabsorption of fluid from the uterine lumen under the influence of progesterone presumably facilitates the reduction in volume of the uterine cavity, preceding the attachment reaction, which has been described by Nilsson (1970) and Ljungkvist (1971a, b, c, 1972) as occurring at the time of implantation in the mouse and in the rat. These workers have shown that implantation involves endometrio-endometrial contact and fusion as well as blastocyst-endometrial contact and fusion. Pollard & Finn (1972) have shown in mice that progesterone alone causes closure of the uterus but, in the absence of oestrogen, it is only the first stage of closure with simple apposition of opposing epithelial cells and interdigitation of the microvilli on their surfaces.

It is not known whether an attachment reaction occurs in man, but Clemetson, Kim, De Jesus, Mallikarjuneswara & Wilds (1973) found the mean uterine fluid volume to be much less in the luteal phase of the menstrual cycle than in the follicular phase. Datnow (1973) also argues that the term 'secretory endometrium', as applied to the luteal phase of the cycle, may not be appropriate. The luteal phase of the menstrual cycle has been known as the secretory phase because of the mucoid material which is seen in the lumen of the endometrial glands in that phase of the cycle and because of the 'subnuclear vacuoles' which are seen in the endometrial epithelial cells just after ovulation. However, it now appears that a clear fluid is secreted into the lumen of the uterus in the follicular phase and simply becomes visible in the luteal phase as a result of inspissation, when water and electrolytes are reabsorbed, and that the 'vacuoles' are artefactual spaces due to loss of glycogen stores during fixation.

Undoubtedly, one of the major reasons why progesterone is essential in pregnancy is that it prevents secretion of fluid by the decidua. The oestrogens present during pregnancy would cause secretion of fluid beneath the membranes and separation of the membranes from the uterine wall, but the presence of progesterone acting to cause reabsorption of fluid from this space and relaxation of the uterine musculature allows expansion of the fetal membranes.

We acknowledge the contributions made by Galla N. Devi and Lakshmi B. Iyer who carried out many of the preparatory experiments which led up to the work reported in this paper. We are grateful to the Board of Trustees of the Methodist Hospital for financial support; and we also thank Mrs Jo Ann Gallo and Mrs Grace Mongiello for their careful work in typing the manuscript and table.

References


REZABEK, K. (1972) The opposite effects of low and high doses of progesterone on the secretion of intrauterine fluid in rats. Physiologia bohemoslov. 21, 137–141.


Received 12 March 1976