Action of Rat Uterine Secretion on the Corona Cells of the Ovum and its Modification by Sex Hormones

SWYER[1] demonstrated in 1947 that the oviduct of the rabbit contains a factor that leads to complete dispersion of the corona radiata of ova within 3 h of their being placed in the fallopian tube. In 1953, SHETTLES[2] produced complete denudation of the human tubal ovum in vitro after incubation at 37 °C for 3 h in homologous blood serum with semen and homologous tubal mucus added. In 1964, MASTROIANNI[3] obtained similar results in vitro when ova from the rabbit oviduct were incubated with secretion from rabbits in oestrus.

These and other similar experiments indicate that the oviduct mucosa or its secretion contains a factor responsible for the liberation of the ovum after normal ovulation when it is surrounded by follicular cells. The object of our experiments was to determine whether the uterine secretion of the rat has the same action in vitro on the cells of the corona radiata and whether this action is modified when the rat is pretreated with sex hormones.

Rat uterine secretion was obtained in the following way: female rats weighing approximately 200 g were primed with oestradiol in a daily dose of 3 µg/kg after excision of the ovaries and oviduct; the uterine secretion was allowed to accumulate for 21 days after ligature of the neck of the uterus. From the 14th to the 21st day, sex hormones were administered s.c. in varying doses, one untreated group of rats serving as controls. On the day of autopsy, which was carried out under sterile conditions, the uterine fluid from all the animals in the same groups was recovered and pooled[4].

The ova were obtained by removing the oviduct from a normal rat in oestrus. Under a lens with a magnification of 16, we opened the oviduct and extracted the freshly ovulated ova. These are surrounded by a mass of follicular cells in a mucous clot. The ova were placed separately, under sterile conditions, in a small, closed chamber containing uterine fluid and incubated for 6 h at 37 °C. Immediately thereafter, and then at hourly intervals, we photographed each ovum, traced the surface projection (× 100) and measured it planimetrically (Figure 1).

The results have been calculated on the basis of the differences in the mean planimetric values of the eggs and corona cells before and after incubation. These values have been expressed in mm².

Results. After 2–3 h incubation in uterine secretion from control rats, the corona cells became detached from the zona pellucida, and after 6 h the surface area had increased by more than 150%, owing to dispersion of the entire corona (Figure 2). Uterine secretion retains this property after freezing, heating to 50 °C and lyophilization.

Upon incubation of the ova with uterine secretion from rats pretreated with oestradiol (3–30 µg/kg, daily), the corona cells showed the same response as the controls (Table I). When ova were incubated in uterine secretion from rats pretreated with progesterone in a dose of 3 mg/kg daily, the dispersion of the corona cells was similar to that observed after incubation in the secretion from control animals; but in secretion from rats pretreated with doses of 10 and 30 mg/kg daily, dispersion was completely inhibited. Incubation in uterine secretion from rats pretreated with 3 and 10 mg/kg testosterone daily gave the same results as incubation in the secretion from control rats, but in the secretion obtained after pretreatment with 30 mg/kg daily, the same degree of inhibition was produced as with 10 mg of progesterone.

It may be concluded that: (1) uterine secretion contains a factor similar to that found in the oviduct by other authors, which induces dispersion of the cells of the corona and liberation of the ovum; (2) treatment with progesterone in doses of 10 mg/kg daily or with testosterone in large doses inhibits the action of this factor, whereas oestradiol in doses up to 30 µg/kg daily is without effect.

Discussion. This phenomenon can be related to modifications of certain physical properties of the uterine secretion induced by large doses of progesterone and testosterone that we observed in previous experiments[4]. It was noted that inhibition of the factor facilitating the dispersion of the cells of the corona radiata takes place when the volume and pH of the secretion are diminished and its viscosity increased (Figure 3). Recently, STAMBAUCH et al.[5] using rabbit oviduct secretion demonstrated that this factor, which acts on mature tubal ova but not on follicular ova, is the bicarbonate ion, since corona cell dispersion can be inhibited by acetozolamide (Diamox). As our experiments were carried out in another species using secretion collected from different sites, it still remains to be seen whether these observations can be confirmed in respect of the effects of uterine secretion on tubal rat ova.

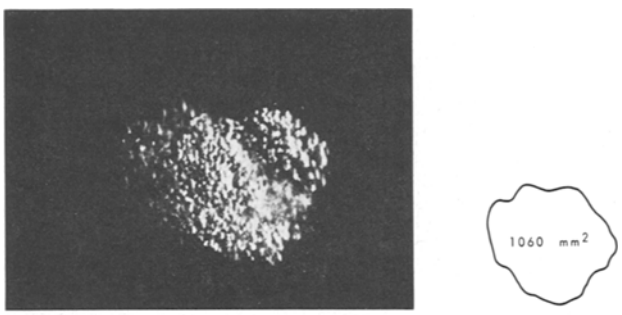

Fig. 1. (A) Rat ova surrounded by corona radiata after ovulation; × 125. (B) Surface area projection; × 100.

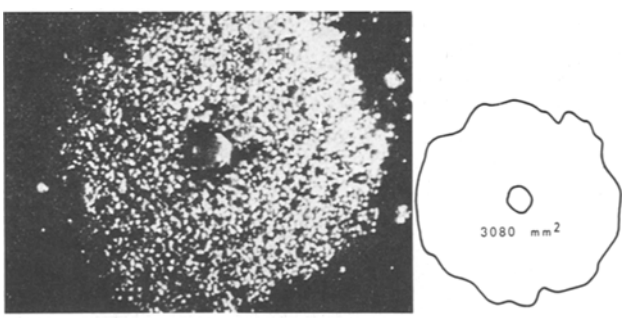

Fig. 2. (A) Ova surrounded by corona radiata after incubation for 6 h in uterine secretion of control rats; × 125. (B) Surface area projection; × 100.

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Difference in surface area of the corona cells of ova after incubation for 6 h in uterine secretion from rats pretreated with oestradiol, progesterone or testosterone

Substances	Doses s.c.	No. of assays No. of rats	Initial surface area (mm^2)	Surface area after 6 h incubation (mm^2)	Absolute increase (mm^2)
Controls	Vehicle only	12/36	1841 ± 108	4708 ± 348	2867 ± 377
Oestradiol	3 µg/kg	2/4	1191 ± 073	3509 ± 490	2318 ± 344
	10 µg/kg	2/6	1233 ± 212	3202 ± 1200	1968 ± 988
	30 µg/kg	2/6	1394 ± 048	3989 ± 076	2495 ± 159
Progesterone	3 mg/kg	2/6	2319 ± 020	4665 ± 561	2346 ± 582
	10 mg/kg	7/19	1401 ± 179	1664 ± 306	264 ± 209
	30 mg/kg	4/10	1254 ± 323	1145 ± 262	− 109 ± 082
Testosterone	3 mg/kg	3/8	1771 ± 056	4071 ± 240	2300 ± 184
	10 mg/kg	3/8	1697 ± 155	4823 ± 780	3125 ± 830
	30 mg/kg	3/8	1437 ± 376	1861 ± 910	424 ± 560

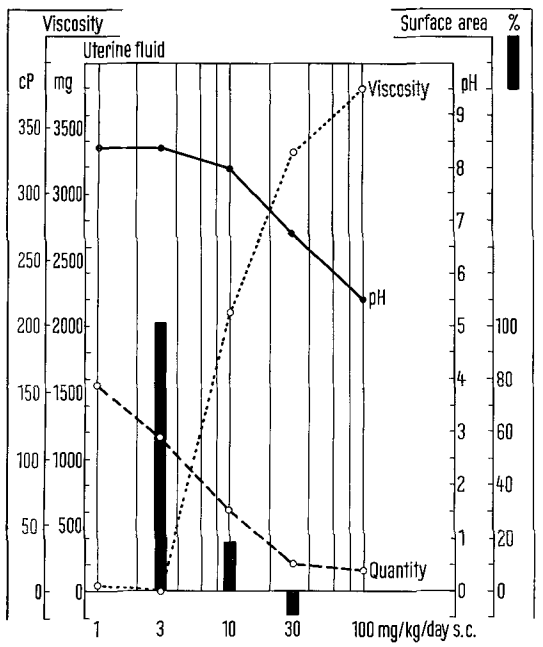

Fig. 3. Physical properties of uterine secretion from progesterone pretreated rats and action in vitro on dispersion of corona cells of the ovum.

PINCUS[6] and MCCLEAN et al.[7] assumed that this factor might be an enzyme similar to spermatozoal hyaluronidase. We have observed that the dispersion of the corona cells can be accelerated in vitro following addition of rat spermatozoa, which suggests that the factor responsible for this effect might well be of an enzymatic nature and that it can be inhibited by progesterone treatment.

Résumé. La ligature des extrémités distales des cornes utérines de rates castrées et sensibilisées à l'œstradiol (3 µg/kg/jour, s.c.) permet de recueillir au bout de 21 jours la sécrétion utérine accumulée. Celle-ci renferme un facteur provoquant in vitro la dispersion des cellules de la couronne de l'œuf. Cette action est inhibée par un prétraitement de progestérone (10 mg/kg/jour, s.c.) du 14è au 21è jour ou par de fortes doses de testostérone. L'œstradiol, par contre, ne présente pas cette action inhibitrice.

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Thyrocalcitonin and Citric Acid

The relationship of calcium and citric acid has been known for a long time[1]. A calcium-citrate complex in blood represents the non-ionized ultrafiltrable fraction of calcium and the amount of citric acid in serum may influence the quantity of ionized calcium. A whole number of hormones and other factors which induce changes of the serum calcium level can influence the citric acid too: parathormone, vitamin D, thyroxin, etc. Thyrocalcitonin (TC) reduces the serum calcium very quickly, and vice versa the production of the hormone is controlled by the actual quantity of calcium in the circulation. So far nothing is known about a possible action of TC concerning organic acids in general and citric acid in particular.

In our experiments we tried to establish whether the level of citric acid follows the changes of the serum calcium and whether it depends on time and dosage. TC was gained from the thyroid glands of pigs according to HIRSCH[2]. The purified extract in acetate buffer (pH 3.8) was injected i.m. to intact 50-day-old male rats of the Wistar strain (100–120 g) which were kept for 4 days on a calcium-free diet. All experimental and control animals

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