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Alterations in intercellular junctions of the uterine epithelium during the preimplantation phase in the rabbit*

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Summary. In the rabbit, the pseudopregnant uterus has been used as a model for studying alterations characteristic of the preimplantation phase. Alterations in intercellular junctions of the uterine epithelium were investigated during early pseudopregnancy (day 0 to day 6) by means of the freeze-fracture technique.

In the uterine epithelium of oestrous females the zonula occludens belongs to the "tight" type of tight junctions. During pseudopregnancy an impressive proliferation of tight junctional belts can be observed. The basal strands proliferate, forming loops perpendicular to the luminal surface, whereas the more or less parallel arrangement of the luminal strands is maintained. At day 4 of pseudopregnancy macular tight junctions begin to develop on the lower portions of the lateral plasmalemma and are extensive by day 6 post hCG.

Small gap junctions are infrequent between cells of the uterine epithelium and show no significant changes during the preimplantation phase.

The physiological significance of the present morphological observations is discussed in the light of changes occurring during the preimplantation period.

Key words: Uterine epithelium – Preimplantation phase – Tight junctions – Gap junctions – Freeze-fracture – Rabbit

It is well established that ovarian hormones control morphological and biochemical changes of the uterine mucosa (Beier and Kühnel 1973, 1976). In the rabbit, hCG triggers ovulation and induces pseudopregnancy, which leads to the same impressive transformation of the uterine epithelium as in pregnancy (Davies and Hoffman 1973, 1975; Beier and Kühnel 1973, 1976; Suzuki and Tsutsumi 1980). This experimental condition is used as an appropriate and convenient model for studies on the physiology of preimplantation. During the preimplantation phase the epithelial cells undergo intense proliferation and ultrastructural change, leading to an intrauterine environment well-adapted for the developing embryo.

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The membranes of epithelial cells are often highly specialized and modified depending on their relationship to neighbouring cells. These differentiations, i.e., the cell contacts such as desmosomes, tight and gap junctions, alter their configurations during different physiological states and during pathological processes (Weinstein et al. 1976; Robenek et al. 1980a, b, c). Observations on ovarian follicular (Merk et al. 1972; Decker 1976; Burghardt and Anderson 1981) and myometrial cells (Dahl and Berger 1978; Garfield et al. 1980) demonstrate hormonal regulation of cell coupling by an increase or diminution in gap junctions.

The size and distribution of tight junctions, which are known to control ion fluxes across epithelial cells, vary according to the functional state (Pitelka et al. 1973; Tice et al. 1975) and can be modified by hormones (Murphy et al. 1980) and pharmacological agents (Robenek et al. 1980a). Since the uterine epithelium undergoes dramatic alterations, under hormonal influence, during transformation into a symplasm during pseudopregnancy, it is of interest to determine what ultrastructural changes occur in epithelial cell junctions during early pseudopregnancy.

Materials and methods

Sexually mature female rabbits (mixed breeds) 3.0–4.0 kg in weight were kept isolated in cages. In order to induce pseudopregnancy, 75 I.U. of human chorionic gonadotropine (hCG) were injected in the ear vein. At different times following injection (day 0 of pseudopregnancy), i.e., 1, 2, 4, and 6 days of pseudopregnancy, the uterus was removed after perfusion fixation. Two rabbits at each phase of pseudopregnancy were investigated. For fixation, rabbits, pretreated with 0.1 ml Regitin (i.m.), were anaesthesized with sodium-pentobarbital (about 0.4 ml/kg) and perfused through the aorta thoracica with 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.3–7.4) for 10 min at room temperature. Both mesometrial and antimesometrial specimens of the endometrium were postfixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer. Following incubation in 20% glycerol in 0.1 M cacodylate buffer overnight, the specimens were frozen in liquid nitrogen-cooled propane and stored in liquid nitrogen.

Replicas were obtained from a freeze-fracture unit (Bioetch 2005, Leybold Heraeus) and examined in a Zeiss EM 10A at 60–80 kV. Morphometry was performed by use of the MOP system (Kontron GmbH).

Results

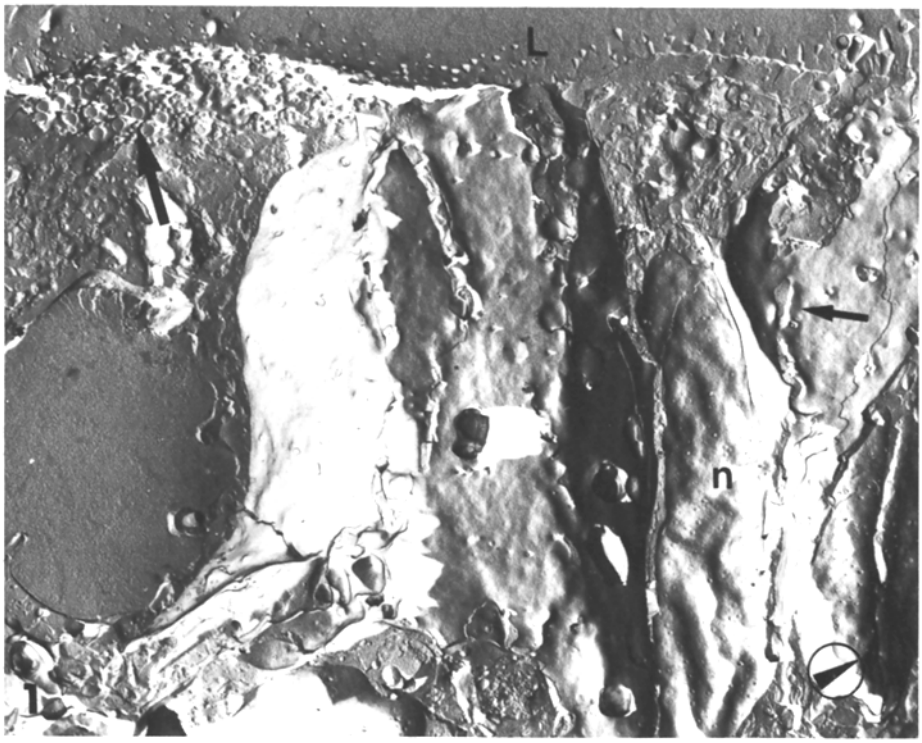
Freeze-fracture replicas of the uterine epithelium of an animal in the oestrous phase confirm the observations of Davies and Hoffman (1975) as well of Busch et al. (1975), and Kühnel and Busch (1981) who investigated the uterine mucosa by use of thin sections and/or scanning electron microscopy.

The luminal epithelium consists of columnar cells, the large nuclei of which display small infoldings. The cell surface is covered with short but regularly arranged microvilli; ciliated cells are intercalated (Fig. 1).

Except for occasional large interdigitating folds, the configuration of the lateral plasmalemma is relatively simple (Fig. 1).

Tight junctions

The epithelial cells are connected by a prominent zonula occludens separating the luminal from the basal side. Tight junctional belts appear as branching and



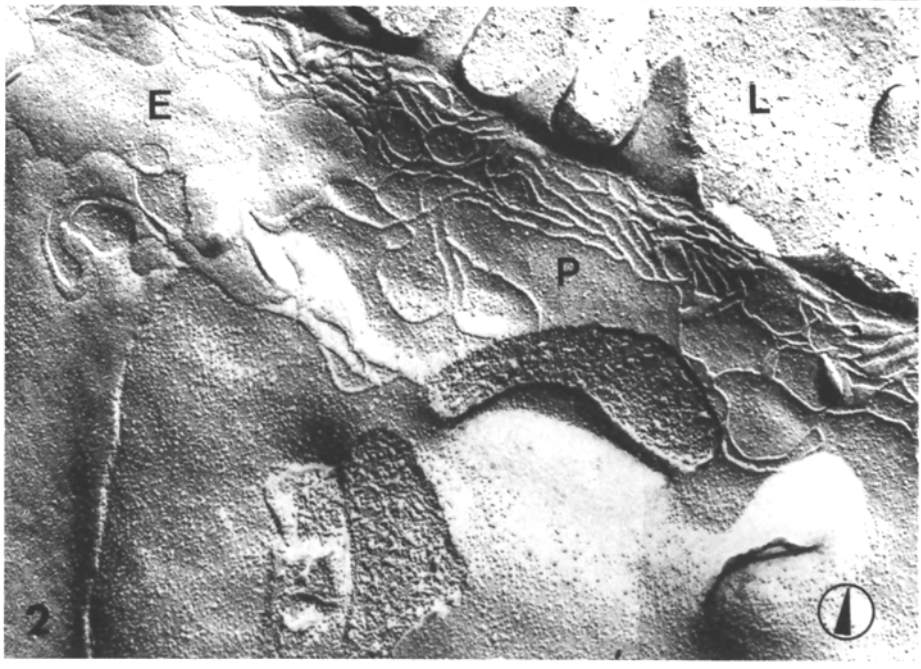


Fig. 1. Freeze-fracture replica of uterine epithelial cells in a rabbit during the oestrous phase. The luminal cell surface is covered with microvilli; ciliated cells are intercalated (*large arrow*). The lateral plasmalemma shows some interdigitating folds (*thin arrow*). Large nuclei (*n*) with small infoldings can be observed. The *encircled arrow* indicates the direction of shadowing in all micrographs. *L* lumen of the uterus. ×6,600

Fig. 2. Tight junctions between uterine epithelial cells in an oestrous rabbit. The strands are arranged more or less parallel to the luminal surface; the abluminal strands form loops. The strands on the P-face are mostly continuous with complementary furrows on the E-face. *L* lumen of the uterus. ×51,200

anastomosing ridges on the P-face and as complementary furrows on the E-face (Fig. 2). The junctional complex is composed of about five to seven strands, which run more or less parallel to the uterine lumen, anastomosing with each other in an irregular pattern. The abluminal strands form loose loops (Fig. 2).

During the first phase of mucosal transformation (day 0 to day 2 of pseudopregnancy) tight junctions increase in number, but vary somewhat in extent (compare Fig. 3a with Fig. 3b). The luminal strands remain unchanged, whereas the lower strands expand covering the lateral plasmalemma in the form of a network (Fig. 3).

The remarkable process continues, and at day 4 of pseudopregnancy most of the epithelial cell membranes show an extensive belt consisting of strands arranged in a meshwork-like fashion (Fig. 4). The continuous abluminal strands form loops that often encircle patches of desmosomes (Fig. 4). During the process of proliferation (day 2 to day 4 of pseudopregnancy) some of the newly formed ridges show free ends. At about day 4 following injection of hCG single strands, detached from the tight-junctional belt, can be observed on the lower portion of the lateral plasma membrane (Fig. 7), forming small macular tight junctions.

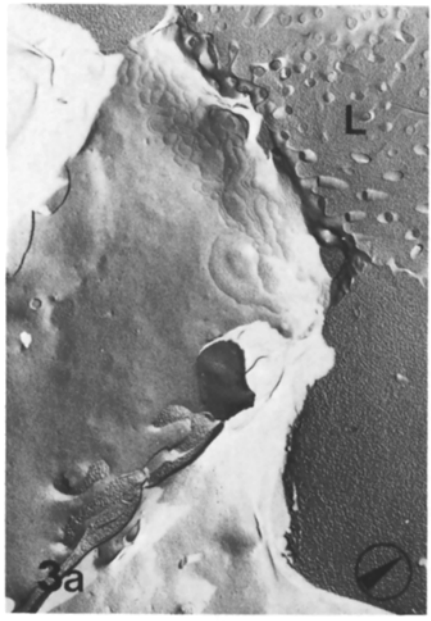
Especially these isolated strands are partly composed of intramembranous particles and sometimes contain small aggregates of particles (see Fig. 7) similar to gap junctions. Beginning at day 6 the uterine epithelium is transformed into multinucleated cells, most probably by fusion.

During this period the tight-junctional area, especially the macular tight junctions, have expanded enormously (Figs. 5, 6). The abluminal protein chains are mostly oriented perpendicular to the luminal surface, whereas the arrangement and direction of the luminal strands are maintained. The pattern of the strands is the same as described at day 4 following hCG injection, with whorl-like loops encircling prominent patches of desmosomes (Fig. 6). On the lateral plasmalemma enlarged macular tight junctions with densely packed strands are generally observed (Figs. 5, 8).

Estimations of the proliferation rate of the zonula occludens in the uterine epithelial cells from oestrus to day 6 of pseudopregnancy are summarized in Table 1. Measurements (random tests) of belts and chains in oestrus vs. day 6 pseudopregnant animals indicate that the membrane area covered with tight junctions is increased approximately seven-fold in the latter. The total length of the protein chains in this area, increasing to about five- to seven-fold, shows higher variation. From these data we can estimate that on day 6 of pseudopregnancy about 20% of the lateral plasma membrane is covered by the tight-junctional belt.

Fig. 3a, b. Tight-junctional belts of uterine epithelial cells at day 2 of pseudopregnancy. The proliferated strands form a meshwork towards the basal side of the cells. Comparison of Fig. 3a with Fig. 3b indicates that the extent of the proliferation varies during this phase of pseudopregnancy. *L* lumen of the uterus. ×1,300

Fig. 4. Tight junctions of uterine epithelial cells at day 4 of pseudopregnancy. The higher magnification reveals that during proliferation the direction of the luminal strands is maintained, whereas the abluminal chains are oriented perpendicular to the cell surface forming loops, which encircle patches of desmosomes (*D*). *L* lumen of the uterus. ×73,300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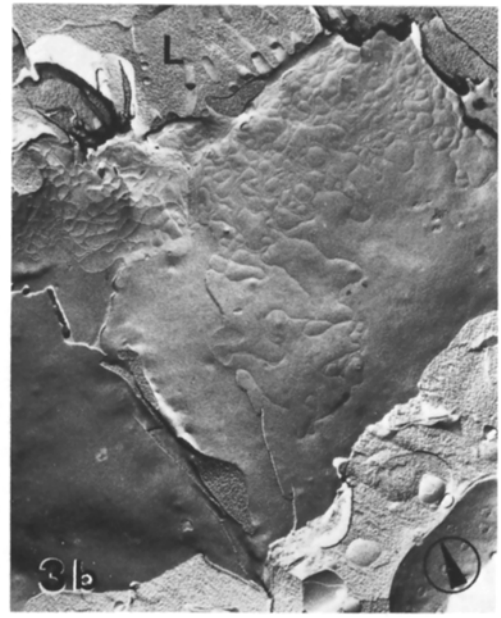
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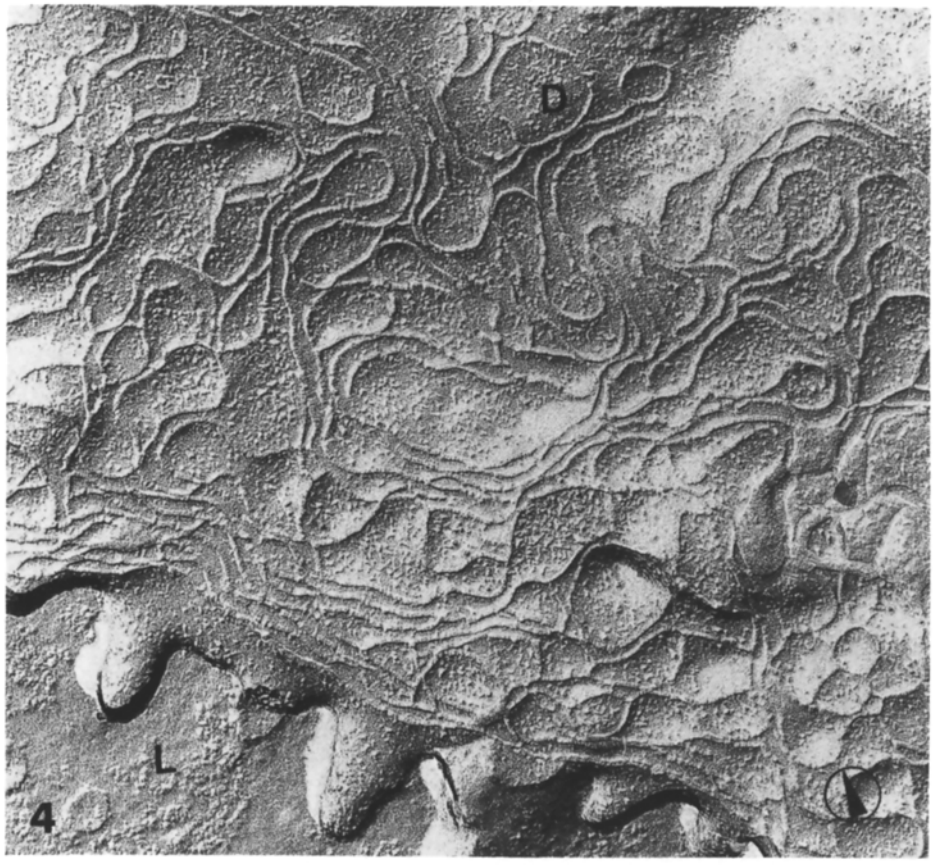
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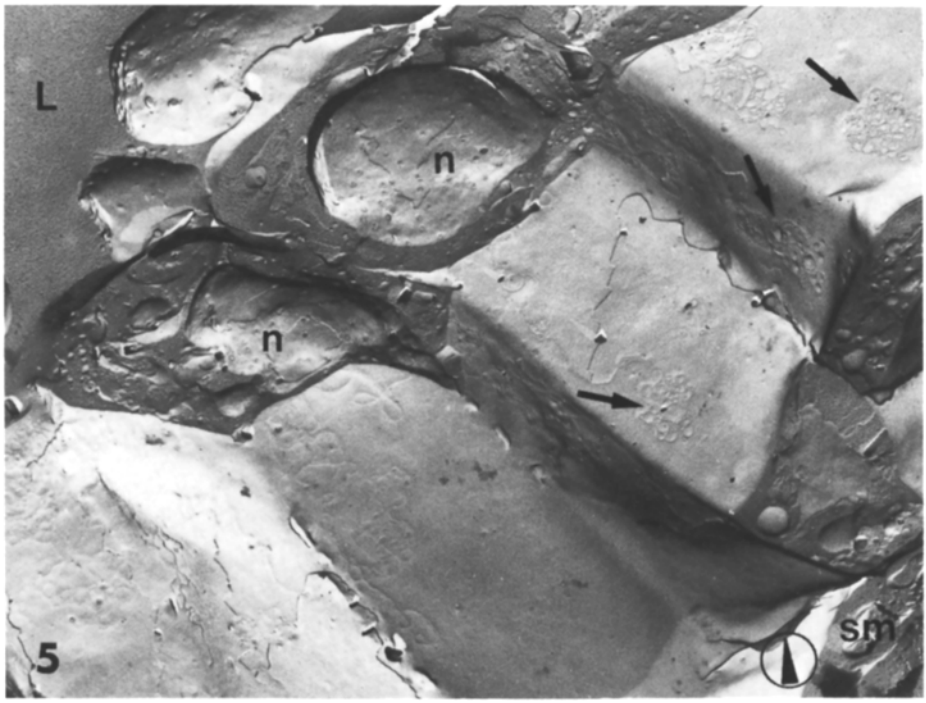

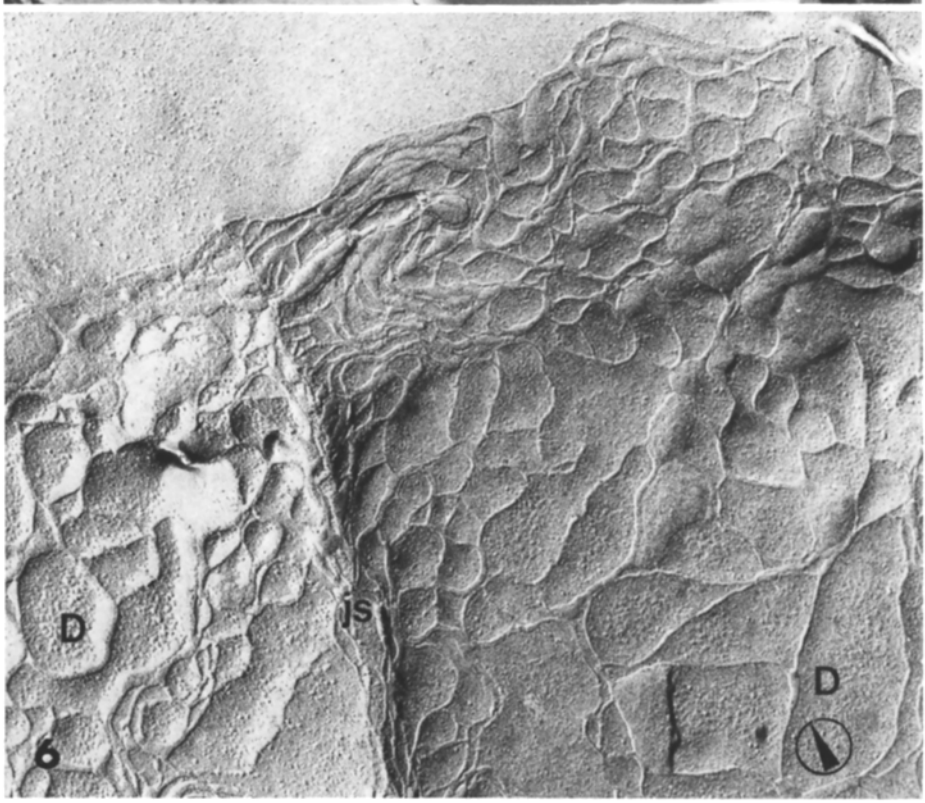

Fig. 5. Uterine epithelial cells at day 6 of pseudopregnancy. The proliferated tight-junctional strands form large macular tight junctions (*arrows*) extending to the basis of the cell. *L* lumen; *n* nucleus; *sm* submucosa. ×8,900

Fig. 6. The luminal belt of the zonula occludens of the uterine epithelium in a 6-day pseudopregnant animal remains similar to that of a 4-day pseudopregnant animal but is larger in extent and exhibits more tight-junctional elements. The abluminal loops encircle patches of desmosomes (*D*). A junctional specialization (*js*), where three cells meet, can be recognized. ×60,000

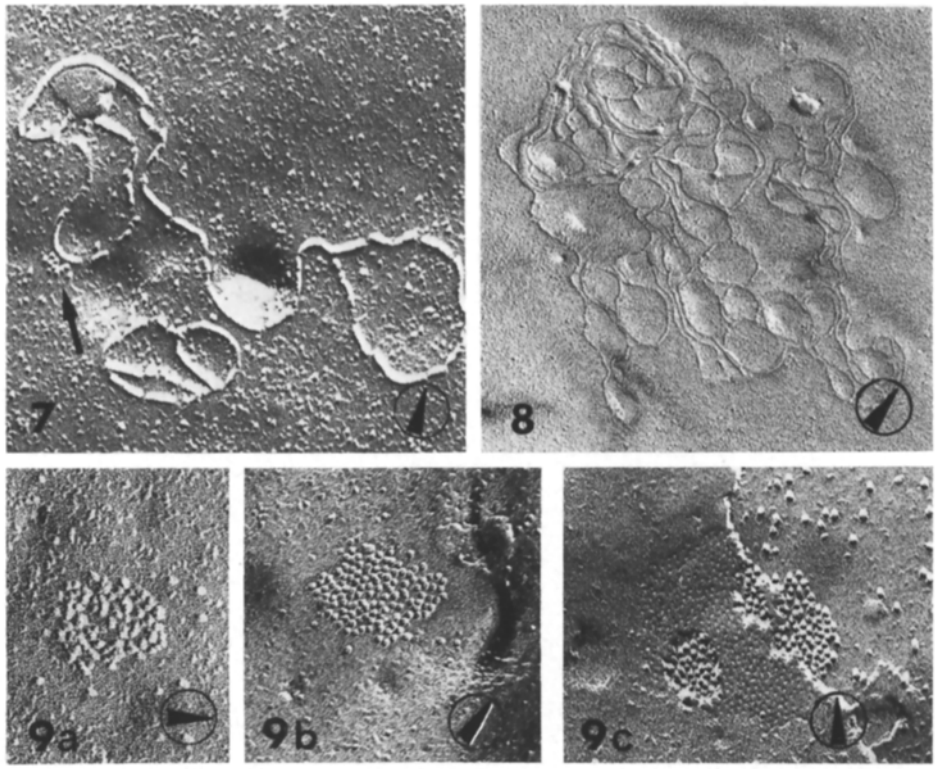

Fig. 7. Macular tight junctions on the lateral cell membrane of the uterine epithelium at day 4 of pseudopregnancy. Note the discontinuous strands, sometimes associated with particle aggregates (*arrow*). ×74,000

Fig. 8. Macular tight junction at day 6 of pseudopregnancy. The macular tight junction is, compared to that described in Fig. 7, larger in extent and contains more tight-junctional strands. ×47,730

Fig. 9a–c. Gap junctions between uterine epithelial cells of an oestrous (**a**) and a 4-day pseudopregnant (**b, c**) rabbit. The gaps are small and consist mostly of loosely aggregated particles. At day 4 and 6 of pseudopregnancy gap junctions with densely packed particles can occasionally be observed (**c**). ×100,000

In some cells about 40% of the lateral plasma membrane areas is covered by tight junctions with large interconnecting macular strands reaching nearly to the base of the cell.

Gap junctions

During the oestrous phase only small and very infrequent gap junctions are found; they consist of loosely aggregated particles about 12–14 nm in diameter (Fig. 9a).

On day 4 of pseudopregnancy gap junctions appear to be more frequent, but are still rare. They are larger in extent and consist of loosely aggregated particles or sometimes of densely packed particles of the same diameter (Fig. 9b–c). The same pattern of gap junctions is observed in the membranes on day 6 of pseudopregnancy, but they are less frequent compared to those of the oestrous phase.

Table 1. Morphometrical comparison of tight junctions in different reproductive phases of the uterine epithelium of the rabbit

Reproductive phase of the rabbit	*n* Animals	*n* Cells	Membrane area[a] covered with tight junctions μm^2	Length of the strands in this area μm^2
Oestrus	2	10	1.75 ± 0.4	42.5 ± 6.2
6 d p. hCG	2	10	12.2 ± 0.3	306.2 ± 109[b]

[a] Membrane area of one side of the lateral plasma membrane
[b] Values of this column vary widely and range from 200 to 500 µm

Discussion

Tight junctions. In every phase of proliferation, the zonula occludens appears to belong to the "tight" type of tight junctions, as classified by Claude and Goodenough (1973) and Claude (1978), with mostly continuous strands corresponding to complementary furrows.

Although direct measurements of the permeability of the uterine epithelium have not been performed, the data would suggest that, with the increase in length of tight-junctional chains, transepithelial resistance may increase during early pregnancy prior to its conversion to symplasm.

Changes in tight-junctional patterns are often governed by the degree of flexibility of the epithelial cells (Hull and Staehelin 1976). Flexibility, however, results more in a reorientation of tight-junctional elements (Hull and Staehelin 1976, Greven and Robenek 1980) than in a proliferation. The impressive proliferation of the tight-junctional belt in the uterine epithelium appears to be dependent on the hormonal state of the rabbit.

There is some evidence that tight junctions in the uterine epithelium of the rat are sensitive to ovarian hormones (Murphy et al. 1980). This study indicates that proliferation occurs during progesterone dominance. However, the zonula occludens of the uterine epithelium of a pregnant and nonpregnant urodele (*Salamandra salamandra*) shows only a slight change in pattern (Greven and Robenek 1980).

Liver tissue, following drug administration, has been used as a model for studying formation and turnover of tight-junctional strands (Robenek et al. 1980 1980a, b, c). Similarly, formation of tight junctions in the uterine epithelium can be studied to advantage following induction by hCG. Based on our observations we agree with the assumption that strands are formed by addition of preexisting or newly synthesized protein particles followed by a subsequent fusion.
Microfilaments may be involved in chain rearrangement (Montesano et al. 1975; Meza et al. 1980; Rassat et al. 1981).

Especially, on day 4 of pseudopregnancy, small clusters of particles similar to gap junctions associated with the strands can be occasionally observed. The combination of the two different types of junctions has been described for several tissues (Elias and Friend 1976; Schiller and Taugner 1979; Murphy et al. 1980; Robenek et al. 1980a). In these tissues, however, the intercalated gap junctions are,

compared to the present material, more extensive. There exists some evidence of a relationship between the formation of gap and tight junctions (Decker and Friend 1974; Albertini et al. 1975; Montesano et al. 1975; Luciano et al. 1979), which suggests a biochemical and functional correlation between these types of intercellular junctions (Schiller and Taugner 1979).

The functional significance of the drastic increase in tight-junctional strands during early pseudopregnancy remains to be determined. It has been suggested, however, that alterations in tight junctions may play a role in regulating uterine fluid volume and composition, thereby influencing the environment of the embryo (Murphy et al. 1980). In addition, formation of tight junctions may be involved in the conversion of the uterine epithelial cells into a symplasm which occurs on day 6 of pseudopregnancy (Davies and Hoffman 1973, 1975; Busch et al. 1981). Proliferation of the tight junctions should help to decrease the intercellular space prior to membrane fusion.

Gap junctions. In contrast to the gap junctions of the myometrium and ovary, gap junctions of the uterine epithelium exhibit relatively little alteration in response to changes in the hormonal environment. The fact that gap junctions are rather uncommon in the uterine epithelium suggest that there is relatively little metabolic and/or electrical coupling. These findings correspond to the observations reported by Greven and Robenek (1980) that the uterine epithelial cells of *Salamandra salamandra* appear to lack gap junctions. The synchronization of proliferation and protein secretion of the uterine mucosa during the preimplantation phase in the rabbit (Beier 1980) does not seem to be governed by cell coupling due to gap junctions.

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