Histochemistry (1987) 87:517–529

Histochemistry

Apical plasma membrane-bound enzymes of rabbit uterine epithelium

Pattern changes during the periimplantation phase

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Received March 10, 1987 / Accepted July 18, 1987

Summary. In order to monitor changes in the apical cell membrane of rabbit uterine epithelium which are postulated to be a precondition for trophoblast attachment, the marker enzymes: alkaline phosphatase, aminopeptidase *M*, γ-glutamyl transferase and dipeptidyl peptidase IV were investigated during the periimplantation phase. Endometrium of early pregnancy (implantation chamber, interblastocyst endometrium; 5–8 days post coitum, d p.c.) was compared with specimens obtained at hCG-induced pseudopregnancy (p. hCG) to distinguish between membrane changes regulated by maternal plasma steroid hormones and such which might be induced locally by blastocyst-derived signals.

All enzymes tested showed their main activity at 5 d p.c./p. hCG. The weakest reaction in this series of stages was generally found at 8 d p.c. (interblastocyst segments) or at 8 d p. hCG. In contrast to the rest of the epithelium, the implantation chamber retained high activity of dipeptidyl peptidase IV, and the activity of alkaline phosphatase even raised here again at 7 and 8 d p.c. indicating a direct local influence of the blastocyst on the luminal epithelium. The results suggest that 1) considerable changes occur in the composition of the apical plasma membrane of the uterine epithelium when the endometrium enters the "receptive state", 2) the overall trend is towards a loss of apical-type characteristics of this membrane domain and 3) the changes are modulated both systemically (by plasma steroid hormone levels) and locally by signals from the implanting blastocyst.

Introduction

In preparation for embryo implantation, the endometrium changes its physiological state in such a way that attachment and, in species with the invasive type of placentation, invasion of the trophoblast is permitted.

Implantation is initiated by an interaction of the trophoblast with the uterine epithelium. In the rabbit, one of the most widely used models for implantation studies, the uterine epithelium undergoes extensive morphological changes during the preimplantation and the periimplantation phase of pregnancy and in the corresponding phases of pseudopregnancy, which are already well documented (Denker 1970, 1977; Enders and Schlafke 1971; Beier 1973; Davies and Hoffman 1973, 1975; Suzuki and Tsutsumi 1980, 1981; Busch 1982). Starting at day 5 p.c./p. hCG, the luminal epithelium forms "dome-like" protrusions of cytoplasmatic compartments which become a characteristic feature for the next day. Just before implantation which occurs at about 7 d p.c., some of the luminal epithelial cells begin to transform into symplasms by fusion and fission of the adjacent lateral cell membranes. This morphological transformation characterizes the so called "receptive state" of the endometrium. The cell biological basis for this state of "receptivity", however, has remained unknown.

There are some reports on changes in the properties of the apical membrane of rabbit uterine epithelial cells during pregnancy and pseudopregnancy: Anderson and Hoffman (1984) noted a loss of surface negativity during the preimplantation period. Nalbach and Denker (1983) showed a change in lectin binding sites and Lampelo et al. (1985) and Anderson et al. (1986) could demonstrate different protein patterns of plasma membranes of receptive and nonreceptive rabbit uterine endometrium. Recent results of Winterhager (1985) revealed an increase in particle density of the apical membranes in this period (also reported for the rat by Murphy et al. (1982)).

This study was performed in order to monitor changes in the apical cell membrane as evidenced by marker enzymes, focussing on the so-called brush-border enzymes: alkaline phosphatase, aminopeptidase *M*, γ-glutamyl transferase and dipeptidyl peptidase IV, on a series of preimplantation stages (5–8 d p.c./ p. hCG).

In contrast to previous studies by Petry et al. (1970) and Kühnel et al. (1971), which have concentrated on differences in various enzymes (including alkaline phosphatase and aminopeptidase *M*, then called leucine aminopeptidase) between the non-pregnant and the 6 d p.c. stage, we were interested specifically in changes of apical vs. basolateral localization characteristics of the enzymes and have com-

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Abbreviations: d p.c. – days post coitum; d p. hCG – days post hCG injection; hCG – human chorionic gonadotropin; aP – alkaline phosphatase; ATPase – adenosine triphosphatase; Ca^{2+}-ATPase – Ca^{2+}-activated adenosine triphosphatase; APM – aminopeptidase M; GGT – γ-glutamyl transferase; DPP IV – dipeptidyl peptidase IV; PCMB – p-chloromercuric benzoate; DFP – diisopropylfluorophosphate; DMF – dimethylformamide

Table 1. *Alkaline phosphatase.* Activity at the apical surface of the uterine epithelium (for different behaviour of deepest parts of crypts see text)

	Pseudopregnancy	Pregnancy		
		Interblastocyst segments	Implantation chamber	
			Paraplacental fold Antimesometrial region	Placental fold
5 d	++(+)	+++	+++	+++
6 d	+(+)	+(+)	+(+)	+(+)
7 d	∅	(+)	++	+(+)
7 1/2 d		∅	+++	+(+)
8 d	∅	∅	+++	+

pared implantation chamber and interblastocyst areas of endometrium as well as endometrium in pseudopregnancy. This allows at the same time to distinguish between membrane changes that are regulated systemically by maternal plasma steroid hormones and such that may be induced locally by trophoblast-derived signals.

Materials and methods

Rabbits were kept and bred as described previously (Denker 1977). Pseudopregnancy was induced by injecting 75 I.U. of human chorionic gonadotropin (hCG) intravenously. Does were sacrified at defined stages post coitum (days post coitum = d p.c., pregnancy) resp. after hCG injection (days post hCG injection = d p. hCG, pseudopregnancy) by stunning and exsanguinating. The uterus was quickly removed, cut into pieces, frozen unfixed in liquid nitrogen and stored at −25° to −30° C in air-tight plastic bags for about 6 to 12 months without loss of activity. One or two rabbits were used for each of the stages of 5, 6, 7, 7 1/2 and 8 days p.c. and of 5, 6, 7, 8 days after hCG injection.

Native longitudinal or cross sections, 14 µm thick, were cut on a cryostat (Dittes-Duspiva, Heidelberg, FRG) at −25° C, mounted at room temperature on glass slides and air-dried for 1–2 min. For alkaline phosphatase the native cryostat sections were mounted on gelatin-coated glass slides, fixed in 4% formaldehyde for 10 min and washed for 10 min in running deionized water in order to restrict diffusion. For the other 3 enzymes we have preferred unfixed sections because localization was sufficiently accurate and because it was of primary interest in these studies to detect even traces of membrane-bound activity while diffusible forms of enzyme were of minor interest. As a control, the histochemical tests were in addition performed on the kidney of the rabbit. A total of 8 enzymes were included in pilot tests, after which 4 enzymes were selected for a complete series of experiments.

The following enzymes were localized by a metal salt procedure:

Alkaline phosphatase – EC 3.1.3.1 (lead method modified after Oledzka-Slotwińska et al. 1967, see Lewis and Knight 1977) substrate: cytidine monophosphate (Boehringer, Mannheim, FRG, No. 103772) (3 m*M*); controls: a) without substrate; b) addition of the inhibitor levamisole (Sigma, Deisenhofen, FRG, L-9756) (0.5 m*M*); pre-incubation (without substrate): 30 min, incubation: 30 min, room temperature.

5′ Nucleotidase – EC 3.1.3.5 (lead technique after Wachstein and Meisel 1957; see Lojda et al. 1979) substrate: adenosine-5′-monophosphate, sodium salt (Boehringer, Mannheim, FRG, No. 102199); controls: a) without substrate; b) addition of the inhibitor levamisole (0.5 m*M*) in order to inhibit alkaline phosphatase; incubation time: 30 min at room temperature.

Adenosine triphosphatase – EC 3.6.1.3 (lead technique modified after Wachstein and Meisel 1957; see Lojda et al. 1979) substrate: adenosine triphosphate, sodium salt (Boehringer, Mannheim, FRG, No 51997); controls: a) without substrate; b) addition of the inhibitor levamisole (0.5 m*M*) in order to inhibit alkaline phosphatase; c) addition of ouabain (Sigma, Deisenhofen, FRG, 0–3125) (10^{-3} *M* resp. 10^{-2} *M*); d) addition of ouabain (10^{-3} *M* resp. 10^{-2} *M*) and levamisole (0.5 m*M*); e) heat-inactivation before incubation (80° C, 15 min); pre-incubation (without substrate): 30 min, incubation: 30 min, room temperature.

Ca^{2+}-activated adenosine triphosphatase (one-step lead citrate method after Ando et al. 1981) substrate: adenosine triphosphate, sodium salt (Boehringer, Mannheim, FRG, No 519979); controls: a) without substrate; b) without activator $CaCl_2$; c) addition of the inhibitor PCMB (Roth, Karlsruhe, FRG, No. 6310) (10 m*M*); d) addition of the inhibitor quercetin (Sigma, Deisenhofen, FRG, Q-0125 (0.1 m*M*); e) heat-inactivation before incubation (80° C, 20 min); incubation time: 30 min at room temperature.

For the other four enzymes a simultaneous azo-coupling procedure was used; diazonium salt Fast Blue B (heavy metal-free; Serva, Heidelberg, FRG, N. 21269):

Aminopeptidase M – EC 3.4.11.2 (arylamidase I, "leucine aminopeptidase", aminopeptidase *N*; simultaneous azo-coupling modified after Nachlas et al. 1960, see Denker and Stangl 1976) substrate: L-leucyl-4-methoxy-2-naphthylamide (Serva, Heidelberg, FRG, No. 27727) dissolved in DMF, addition of 10^{-3} *M* $CoCl_2$ to the incubation medium; incubation time: 30 min at 37° C.

γ-Glutamyl transferase – EC 2.3.2.2 (simultaneous azo-coupling procedure modified after Rutenburg et al. 1969; see Lojda et al. 1979) substrate: γ-glutamyl-4-methoxy-2-naphthylamide (Bachem, Bubendorf, CH) dissolved in DMF and NaOH; addition of glycylglycine (Sigma, Deisenhofen, FRG) as acceptor molecule; control: without glycylglycine; incubation time: 30 min at room temperature.

Aminopeptidase A – EC 3.4.11.7 (simultaneous azo-coupling after Kugler 1981) substrate: α-L-glutamyl-4-methoxy-2-naphthylamide (Bachem, Bubendorf, CH) dissolved in DMF; control: without substrate; incubation time: 30 min at 37° C.

Dipeptidyl peptidase IV – EC 3.4.14.5 (simultaneous azo-coupling after Gossrau 1979) substrate: glycyl-prolyl-4-methoxy-2-naphthylamide (Sigma, Deisenhofen, FRG, free base, No. G 9137) dissolved in DMF; controls: a) without substrate; b) addition of the inhibitor DFP (Serva, Heidelberg, FRG, No. 77206) (1 m*M*); incubation time: 30 min at room temperature.

The sections were thoroughly rinsed in distilled water and embedded in glycerin jelly. Other chemicals used were obtained from Merck (Darmstadt, FRG) and were of analytical grade.

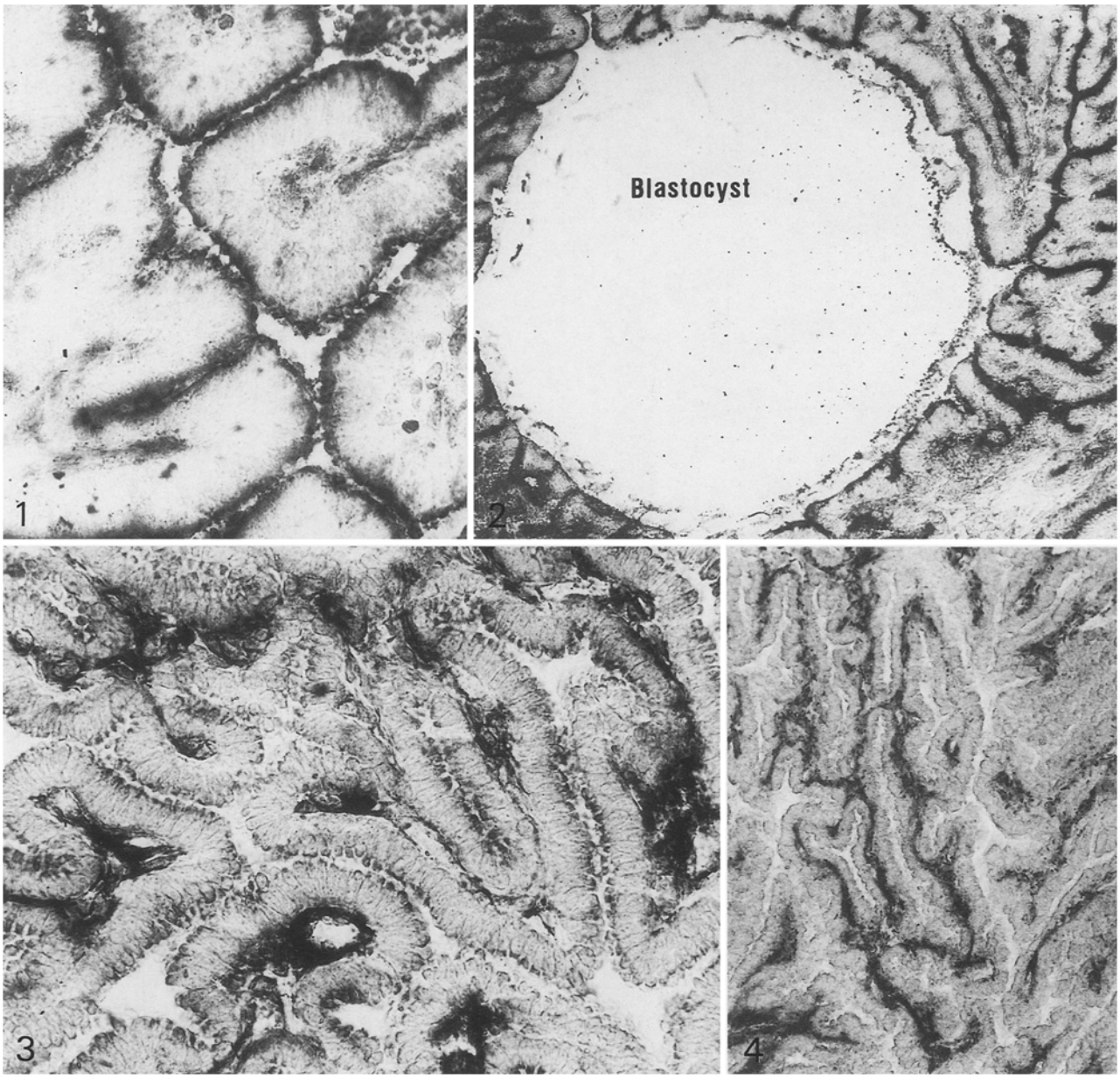


Fig. 1. Alkaline phosphatase, 5 d p.c. A strong reaction is observed mainly at the apical plasma membrane of the uterine epithelium. In this section the subepithelial stroma is only weakly stained; ×350

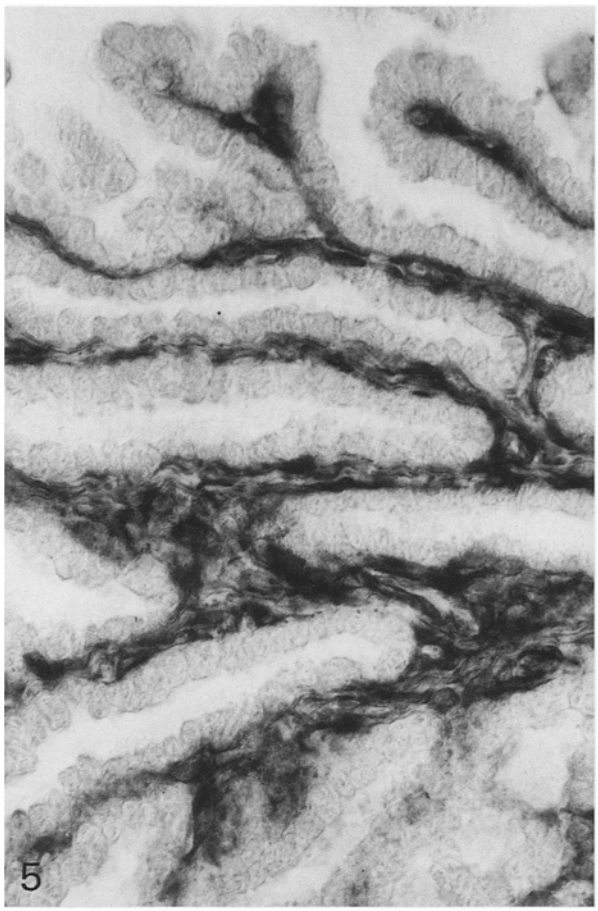
Fig. 2. Alkaline phosphatase, 5 d p.c. Endometrium and blastocyst. Portions of epithelium which are adjacent to the *blastocyst* show the same strong reactivity as more distant parts; ×88

Fig. 3. Alkaline phosphatase, 6 d p.c. Although the epithelial reaction is weaker compared to 5 d p.c., many apical protrusions are clearly stained, the subepithelial stroma shows strong aP activity; ×350

Fig. 4. Alkaline phosphatase, 7 d p.c., interblastocyst segment. The activity of aP is strongly reduced in the apical plasma membrane of the epithelium. Only the subepithelial stroma retains the high aP activity; ×140

Results

In pilot test series, the preimplantation stage 5 d p.c. and the implantation stage 7 1/2 d p.c. were checked for any positive enzyme reaction at the uterine epithelium, for each of the enzymes chosen. On this basis the following enzymes: 5′-nucleotidase, ATPase, Ca^{2+}-ATPase and aminopeptidase *A* were not further investigated at other stages, because they were not useful markers for the epithelium and showed their main activity in blood vessels (endothelium) and in the myometrium (5′-nucleotidase and ATPase showed also a positive reaction in the subepithelial stroma). On contrast, alkaline phosphatase, aminopeptidase *M*, GGT and DPP IV were selected for testing in all stages of pregnancy and pseudopregnancy, since they showed strong reaction at the apical plasma membrane at 5 d p.c./p. hCG.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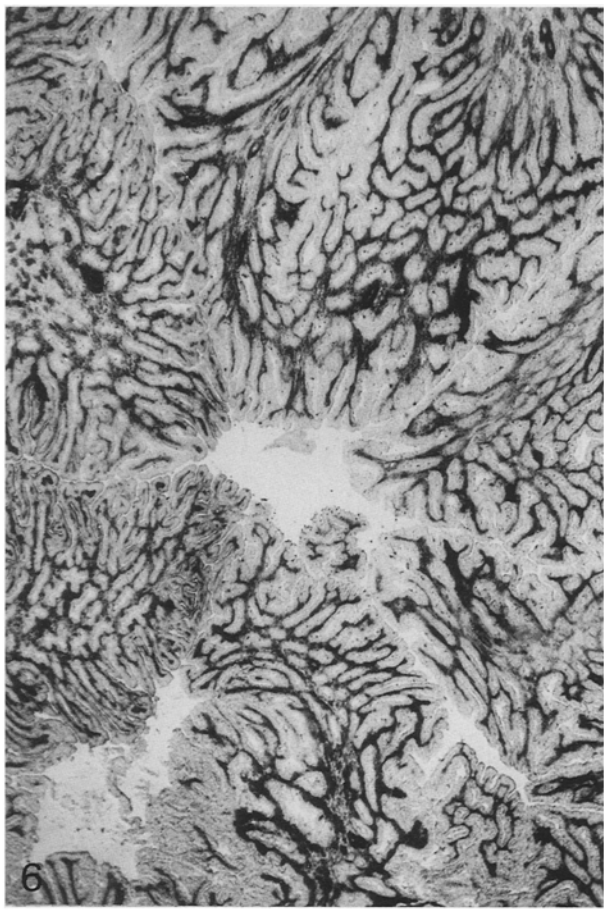
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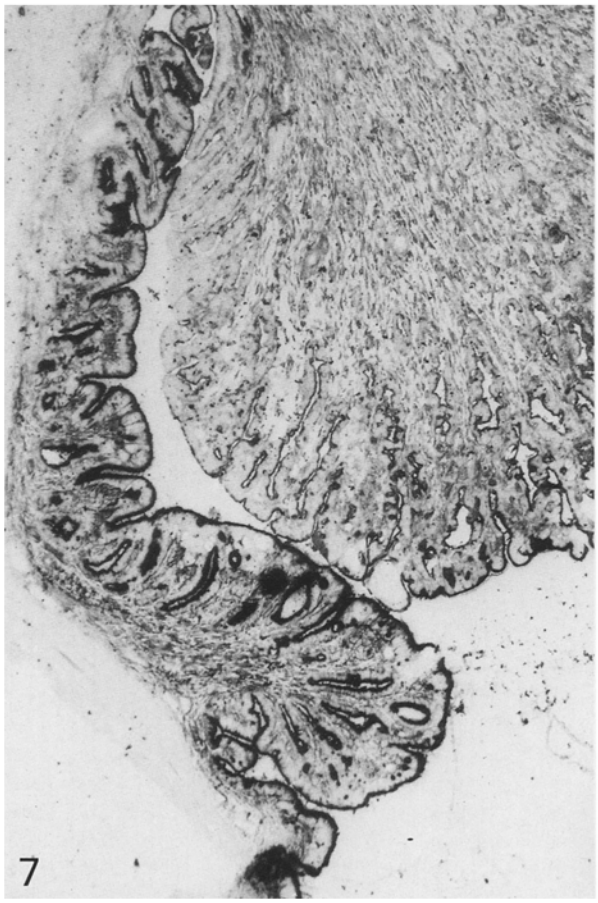
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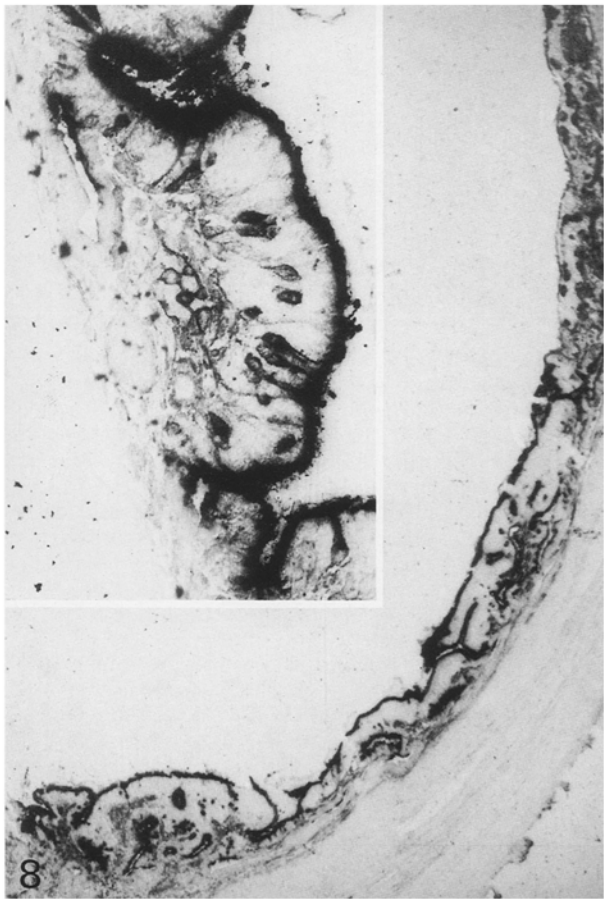
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Table 2. *Aminopeptidase M.* Activity at the apical surface of the uterine epithelium (for different behaviour of deepest parts of crypts see text)

	Pseudopregnancy	Pregnancy		
		Interblastocyst segments	Implantation chamber	
			Paraplacental fold Antimesometrial region	Placental fold
5 d	+++	+++	+++	+++
6 d	∅−++	(+)−++	+	+
7 d	∅	∅	∅	∅
7 1/2 d		∅	∅	∅
8 d	∅	∅	∅	∅

I. Alkaline phosphatase (aP) (see Table 1)

a) Pregnancy (interblastocyst segments)/Pseudopregnancy. At 5 d p.c. (pregnancy) a strong reaction was obtained mainly in the apical region of the epithelium (see Fig. 1). The reaction was quite comparable to the results in pseudopregnancy at 5 d p. hCG, although slightly stronger. The deepest parts of crypts near the myometrium were not stained (compare APM, Fig. 10). At 6 d p.c. (Fig. 3) and just so at 6 d p. hCG the positive apical reaction of the epithelial plasmalemma was reduced as compared to the previous stage. A pronounced activity of alkaline phosphatase was found on the apical membrane at the dome-like protrusions (see Fig. 3).

At 7 d p.c. the apical plasma membrane of the uterine epithelium was considerably less stained (see Fig. 4) and the activity decreased even further at 7 1/2 d p.c. (see Fig. 5) and 8 d p.c. (see Fig. 6). In pseudopregnant animals a slightly different time course of declining was observed: At 7 d p. hCG the positive reaction had already disappeared. The 8 d p. hCG stage was again comparable to the 8 days pregnant stage.

b) Pregnancy (implantation chamber). At 5 and 6 d p.c. there was no difference between the reaction of the uterine epithelium surrounding the blastocyst and the epithelium of interblastocyst segments (see Fig. 2). A difference became first detectable at 7 d p.c. and became more pronounced at 7 1/2 d p.c., when the luminal epithelium in the implantation chamber was significantly more intensely stained than the other areas (compare Fig. 5 with Figs. 7 and 8). In the implantation chamber, the reaction was stronger at the antimesometrial side (former obplacental folds) and at the paraplacental folds as compared to the placental folds (see Fig. 7), where the reaction was weak and decreased even further until 8 d p.c..

Besides the apical membrane of the uterine epithelium, the subepithelial stroma (region of the subepithelial capillary plexus) showed a strong but diffuse aP reaction in all stages, whereas the rest of the stroma and larger blood vessels were only slightly stained.

The control sections incubated without substrate or with the inhibitor levamisole showed no reaction in the epithelium and in the subepithelial stroma. Levamisole did not suppress completely the aP activity in the myometrium and at larger blood vessels at 7 and 8 d p. hCG and in the control organ kidney.

II. Aminopeptidase M (APM) (see Table 2)

a) Pregnancy (interblastocyst segments)/Pseudopregnancy. At 5 d p.c./p. hCG a very strong apical reaction of APM-activity was found in the cavum epithelium and at the upper and medium parts of crypts (see Figs. 9 and 10). No reaction was seen in the deepest parts of the crypts (see Fig. 10). The results concerning the activity of APM at 6 d p.c. were heterogeneous (see Table 2) in different rabbits. APM could clearly be detected in the apical membrane of the uterine epithelium in one animal while in another animal the reaction was rather weak. The same phenomenon, i.e. a different reactivity in different animals was also observed in pseudopregnancy at the same stage. In both cases, in pseudopregnancy as well as pregnancy, the weak reaction was correlated to a more progressed proliferation and transformation of the mucosa (more resembling the 7 d stage, see Davies and Hoffman 1973).

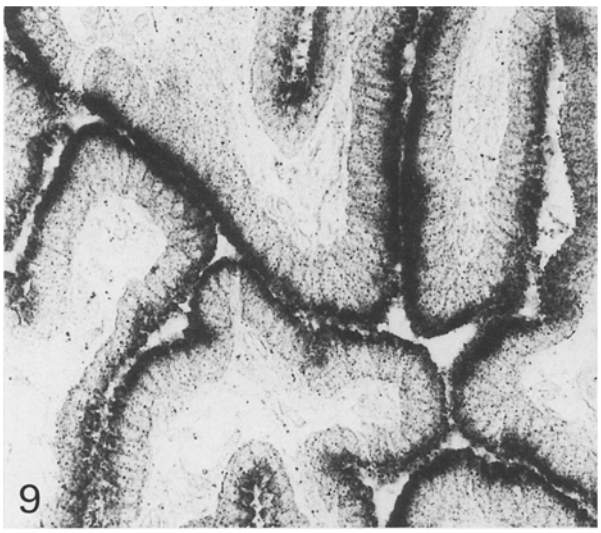
The strong apical reaction had disappeared in all specimens at 7 d p.c. or 7 d p. hCG except for a diffuse weak staining remaining in the whole epithelium. At 7 1/2 d p.c. and 8 d p.c./p. hCG the aminopeptidase *M* activity was totally abolished (see Fig. 11).

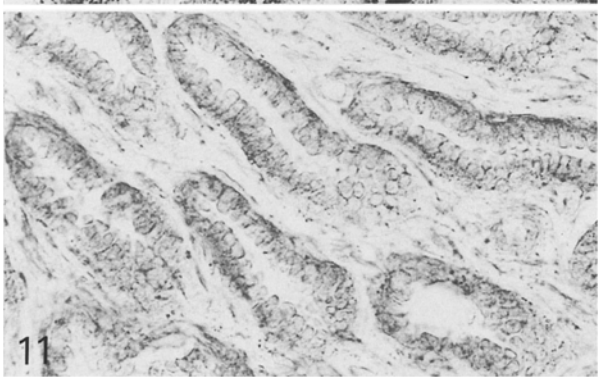
Fig. 5. Alkaline phosphatase, 7 1/2 d p.c., interblastocyst segment. The subepithelial stroma shows the main reaction of aP, while the apical plasmalemma of epithelial cells is nearly negative; ×350

Fig. 6. Alkaline phosphatase, 8 d p.c., interblastocyst segment. Cross-section of the endometrium. The activity of aP in the apical membranes of epithelial cells is completely abolished; ×35

Fig. 7. Alkaline phosphatase, 7 1/2 d p.c. Placental and paraplacental fold next to the blastocyst. A positive reaction of the luminal epitelium is observed predominantly at the paraplacental fold; ×35

Fig. 8. Alkaline phosphatase, 8 d p.c. The antimesometrial luminal epithelium of the implantation chamber shows strong apical staining; ×35; *inset*: ×140, some unidentified cells in the epithelium differ in showing strong cytoplasmic staining





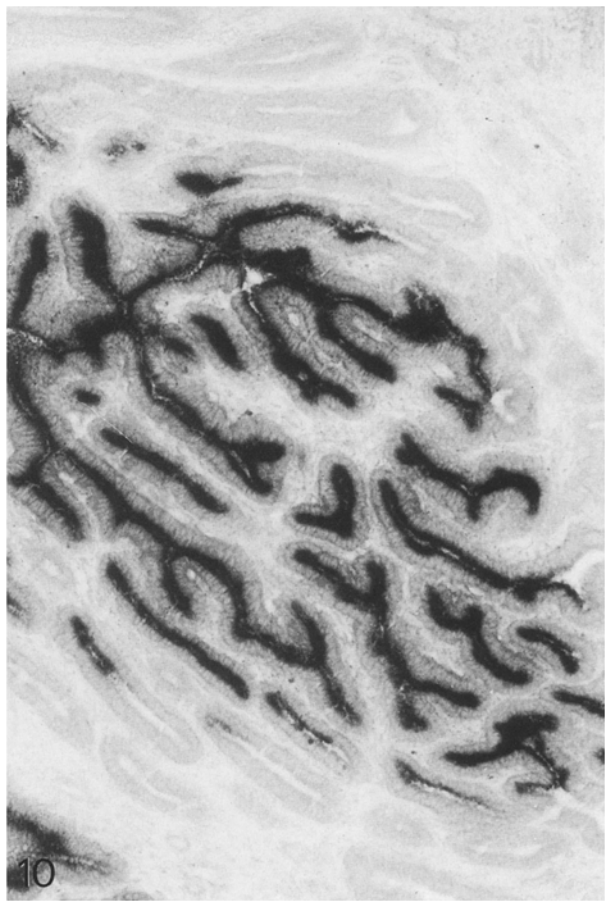


Fig. 9. Aminopeptidase *M*, pseudopregnancy 5 d p. hCG. A strong apical reaction of the uterine epithelium lining the cavum and the middle parts of crypts can be observed; ×350

Fig. 10. Aminopeptidase *M*, 5 d p.c. In deepest parts of crypts no activity of APM is observed; ×140

Fig. 11. Aminopeptidase *M*, pseudopregnancy 8 d p. hCG. No positive reaction in the uterine epithelium can be detected anymore; ×350

Table 3. *γ-Glutamyl transferase.* Activity at the apical surface of the uterine epithelium

	Pseudopregnancy	Pregnancy		
		Interblastocyst segments	Implantation chamber	
			Paraplacental fold Antimesometrial region	Placental fold
5 d	++(+)	+++		
6 d	+++	++(+)	++(+)	++(+)
7 d	+	++	++	+(+)
7 1/2 d		+	+	(+)
8 d	(+)	(+)	(+)	∅

b) Pregnancy (implantation chamber). At 5 d p.c. the apical plasma membrane of the epithelial cells surrounding the blastocyst demonstrated the same strong reaction as in interblastocyst segments. One day later (6 d p.c.), there was a more pronounced decrease of apical staining in the vicinity of the blastocyst compared to interblastocyst segments. At 7, 7 1/2 and 8 d p.c. the clear apical staining was abolished and no difference between blastocyst sites and interblastocyst segments could be discerned.

At all stages myometrium and blood vessels were stained with a main activity at 7 1/2 d p.c.

III. γ-Glutamyl transferase (GGT) (see Table 3)

a) Pregnancy (interblastocyst segments)/Pseudopregnancy. At 5 and 6 d p.c or p. hCG the apical membranes were strongly stained in the cavum epithelium and in upper parts

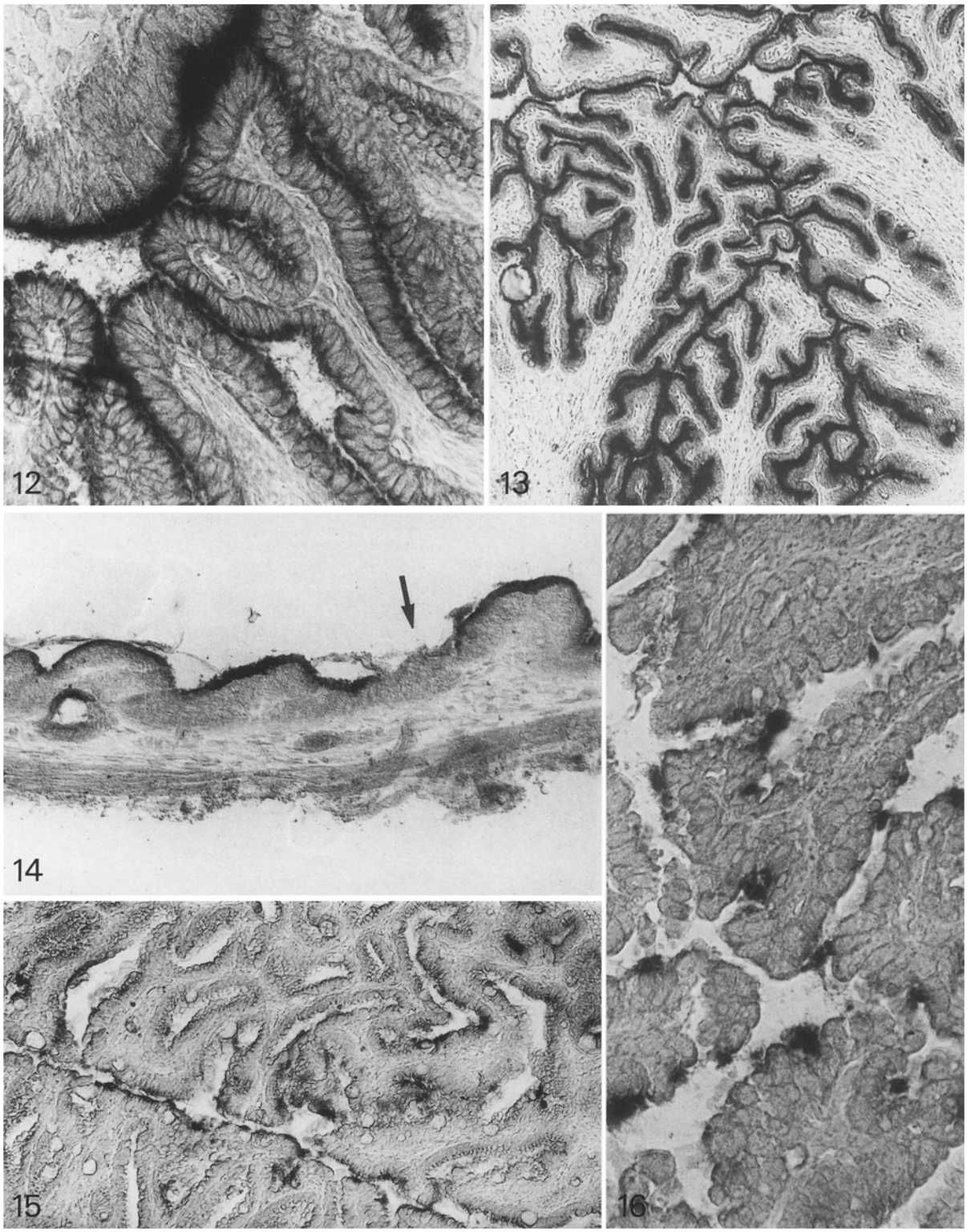


Fig. 12. γ-Glutamyl transferase, 5 d p.c. A strong reaction of the apical membranes of the uterine epithelium is observed; × 350

Fig. 13. γ-Glutamyl transferase, 6 d p. hCG. A strong apical staining, even slightly more intense than at 5 d p. hCG can be seen; × 88

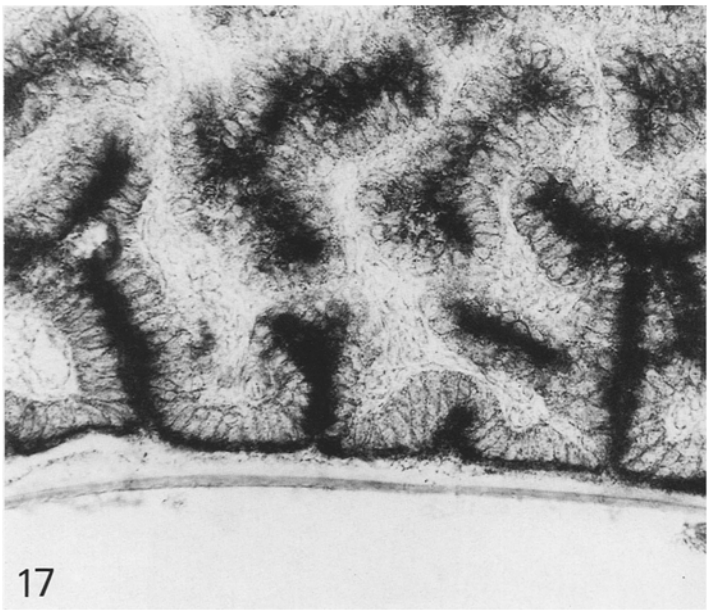
Fig. 14. γ-Glutamyl transferase, 7 1/2 d p.c. Antimesometrial region with an attached trophoblastic knob (*arrow*). The apical membrane region of luminal epithelium shows a positive reaction; × 140

Fig. 15. γ-Glutamyl transferase, pseudopregnancy 8 d p. hCG. The epithelial reaction is largely lost. Remaining activity is predominantly located in the deepest parts of the crypts or at the luminal epithelium; × 140

Fig. 16. γ-Glutamyl transferase, 8 d p.c., interblastocyst segment. Some single cells are still stained, whereas the overall reaction is considerably diminished; × 350

Table 4. *Dipeptidyl peptidase IV.* Activity at the apical surface of the uterine epithelium (for different behaviour of deepest parts of crypts see text)

	Pseudopregnancy	Pregnancy		
		Interblastocyst segments	Implantation chamber	
			Paraplacental fold Antimesometrial region	Placental fold
5 d	+++	+++	+++	+++
6 d	+++	+++	+++	+++
7 d	+++	++	+++	++
7 1/2 d		+	+++	++
8 d	(+)	(+)	+++	+



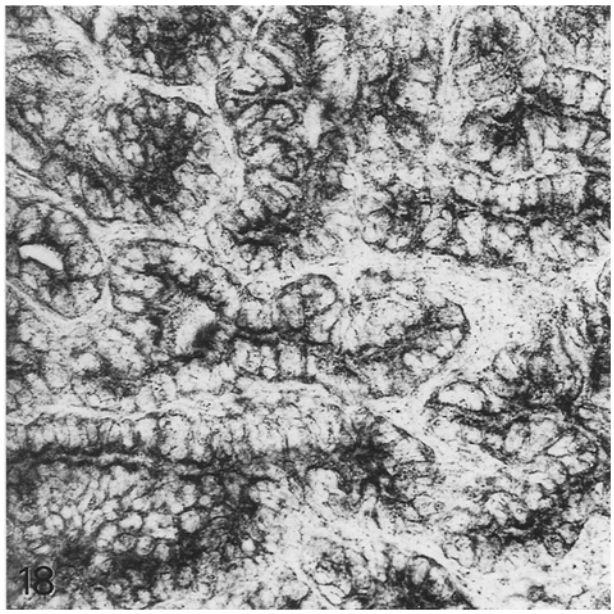


Fig. 17. Dipeptidyl peptidase IV, 6 d p.c. Epithelium surrounding the blastocyst. Note the uniformly strong apical reaction in the luminal as well as in crypt epithelium; ×350

Fig. 18. Dipeptidyl peptidase IV, 7 1/2 d p.c., interblastocyst segment. The staining of the apical plasma membrane of the uterine epithelium is strongly reduced but still visible. The weak staining of the basolateral membranes becomes more apparent; ×350

of the crypts as well as in middle and deep parts (see Figs. 12 and 13). At 7 d p.c., staining was less intense than one day earlier and this reduction was even more pronounced in pseudopregnancy (7 d p. hCG). At 7 1/2 d p.c. and 8 d p.c. or p. hCG only a weak reaction at the apical plasmalemma was left; at 8 d p.c. even less than half a day earlier. A few single cells in the luminal epithelium and in deeper parts of the crypts showed a rest of activity (see Figs 15 and 16).

b) Pregnancy (implantation chamber). The epithelium surrounding the blastocyst showed in all stages the same enzyme activity (i.e. also the same decline) as interblastocyst parts of the endometrium (see Fig. 14) except for the placental fold, which was less stained from 7 d p.c. on.

In all stages no staining of any other histological elements of the uterus besides the epithelium could be observed. Control sections without the acceptor molecule glycylglycine in the incubation medium (see Materials and Methods; Lojda et al. 1979) showed considerable suppression of the reaction. At stages of maximal GGT activity, i.e. at 5 and 6 d p.c. and p. hCG, a very weak positive reaction was obtained even without glycylglycine.

IV. Dipeptidyl peptidase IV (DPP IV) (see Table 4)

a) Pregnancy (interblastocyst segments)/Pseudopregnancy. At 5 and 6 days of pregnancy or pseudopregnancy a strong reaction of the apical plasma membrane of the uterine epithelium was obtained (see Fig. 17). At 7 d p. hCG the reaction was as strong as at the two former stages; on contrast, the staining was slightly reduced at 7 d p.c. In deeper parts of crypts, near the myometrium, the reaction was more intense than in luminal parts. No reaction was seen in the deepest parts of crypts, the difference between both types of epithelial cells being most obvious at 5 and 6 d p.c./p. hCG. At 7 1/2 d p.c. there was still a positive reaction at

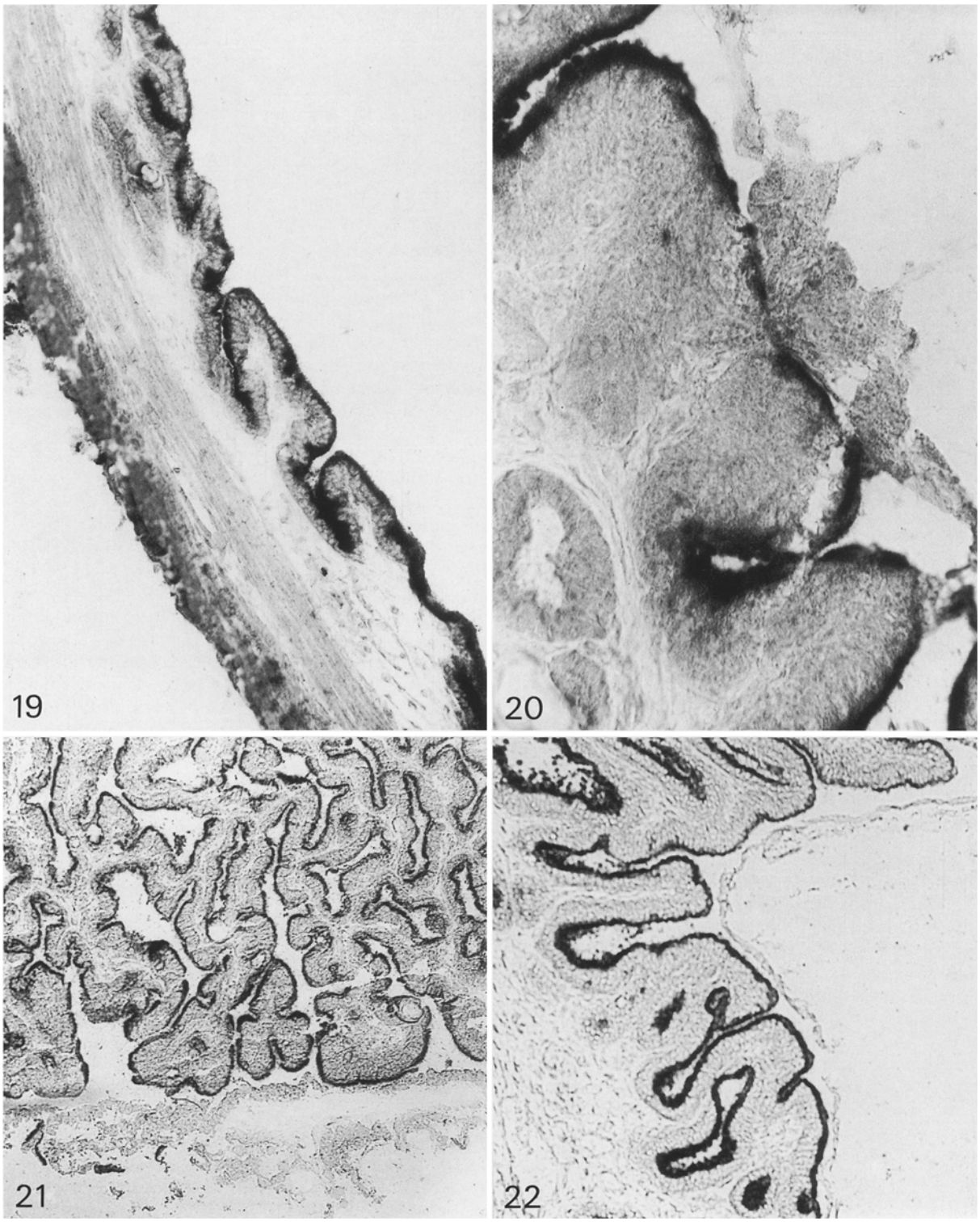


Fig. 19. Dipeptidyl peptidase IV, 7 d p.c. Antimesometrial region of the implantation chamber; there is a strong activity of DPP IV at the luminal epithelium; × 140

Fig. 20. Dipeptidyl peptidase IV, 7 1/2 d p.c. Antimesometrial region of the implantation chamber with an attached trophoblastic knob. The apical plasma membrane of the uterine epithelium is strongly stained except for the area of established attachment; × 350

Fig. 21. Dipeptidyl peptidase IV, 7 1/2 d p.c. Implantation chamber, mesometrial region including blastocyst wall. Less staining than in the antimesometrial region can be noted; × 88

Fig. 22. Dipeptidyl peptidase IV, 8 d p.c. Paraplacental region near the blastocyst; the staining of the luminal epithelium is rather strong; × 88

the apical plasmalemma of the uterine epithelium especially in parts far away from the lumen and close to the myometrium, but the reaction was significantly reduced compared to previous stages (see Fig. 18). At 8 d p.c. or p. hCG only a very weak activity had remained.

b) Pregnancy (implantation chamber). At 5 and 6 d p.c. the luminal epithelium surrounding the blastocyst did not differ from the epithelium in other areas without blastocysts (see Fig. 17). At 7, 7 1/2 and 8 d p.c. the apical staining of the cavum epithelium adjacent to the blastocyst was as strong as at former stages (see Figs. 19, 20, 22) except for the placental fold where it was diminished (see Fig. 21).

As with GGT no staining of any other histological elements besides the epithelium could be observed at any stage. The inhibitor DFP completely abolished the activity of DPP IV.

Discussion

Although the different catalytic functions of the individual enzymes are more or less known, as we will discuss below, their physiological role in the uterine epithelium is not yet understood and, consequently, the biological significance of the observed changes in enzyme patterns cannot be easily explained in relation to the implantation process.

Remarkably, however, our results show that there is a common trend in their behaviour, i.e. progressive loss of activity towards implantation time (with some specific changes in the implantation chamber). We propose, therefore, that the common feature is a profound change in membrane composition which causes these functionally unrelated enzymes to change their activities in a concerted way, since all these enzymes are integral membrane proteins.

Alkaline phosphatase

Alkaline phosphatase – a very ubiquitous enzyme which catalyzes the hydrolysis of esters of orthophosphoric acid with various alcohols and phenols in the alkaline range – is found primarily in cell membranes where active transport processes take place (see Lojda et al. 1979).

In our study a strong activity of aP was found at 5 and 6 days of pregnancy and pseudopregnancy at the apical membrane of the uterine epithelium, which was considerably reduced at day 7 and 8 of pseudopregnancy or in interblastocyst segments. In contrast, the enzyme activity increased again in the implantation chamber at 7 1/2 and 8 days (see Table 1) especially at the antimesometrial side and paraplacental folds.

Our findings extend former investigations of the rabbit uterus by Christie (1967) who described an abrupt increase in enzyme activity in the implantation chamber at the time of trophoblastic invasion (7 1/2 d p.c.), and former studies of Petry et al. (1970) who found a rather strong activity at the apical part of the epithelium at 6 d p.c.

In the uterus of the marmoset and rat aP was localized in the apical cytoplasm of surface and glandular epithelium (Blanke and Graf 1985). Suzuki M. et al. (1981) could show a cycle dependency of aP in human endometrium: the aP activity increased in the proliferative phase and decreased in the secretory phase. The physiologic role of alkaline phosphatase in the implantation process remains unknown, although several functions of the enzyme have been discussed (Christie 1967; Petry et al. 1970; Birge 1981; Izquierdo and Marticorena 1975; Lin et al. 1976).

Aminopeptidase M

Aminopeptidase *M* (also referred to as aminoacid arylamidase I, aminopeptidase *N*, leucine aminopeptidase, leucine naphthylamidase) hydrolyzes various peptides and arylamides, e.g. L-leucyl- and L-alanyl-2-naphthylamide or p-nitroanilides (see Denker and Stangl 1976; McDonald and Barrett 1986).

We found a strong reaction at the apical surface of uterine epithelial cells at 5 days p.c./p. hCG and then a decline of APM activity towards implantation time (about 7 d p.c.). In the implantation chamber the decline occurs already somewhat earlier. These results are in general agreement with previous investigations of Denker (1971) and van Hoorn and Denker (1975). Details concerning the strength of the residual activity at 7 d p.c. might be due to a methodological difference (freeze substitution preserving higher activity as confirmed with the present material, data not shown).

The heterogeneous results in different rabbits at 6 d p.c./p. hCG indicate that this stage is rather critical. The transition from high activity to low activity seems to occur very rapidly. As biochemical measurements of APM in uterine flushings have shown (Denker 1980), the peak activity lasts only for approximately one day in the uterine lumen. Slight phase shifts perhaps occurring in the inferred quick process of release from the epithelium could explain the different results seen in different rabbits. Morphologically these animals had – although sacrificed at the same time after induction of ovulation – a different degree of endometrial transformation.

As Denker (1969) and Kühnel et al. (1971) could show there is no APM-activity in the non-pregnant state and in early pregnancy, but the activity increases at 3 d p.c., has a maximum at about 5 d p.c. and declines thereafter (Denker 1980). The increase of activity of APM has been shown to be progesterone-dependent (Denker 1980), but there is no explanation for the decline at about 7 d p.c. The serum progesterone level starts to decline in pseudopregnancy at about 12 d p. hCG but is constant between 7 and 11 days (see Fischer et al. 1985, for a review of literature). A steroid hormone dependency for APM is also discussed to occur in the marmoset by Blanke et al. (1984) and in the human by Fuhrmann (1959). Although here the predominant localization of the enzyme is in the stroma, there is also a reaction in the luminal epithelium of the endometrium (Fuhrmann 1959; Blanke and Graf 1985).

The enhanced secretion of APM into the uterine lumen during the periimplantation phase in the rabbit (Denker 1980) was discussed in connection with the needs for blastocyst "nutrition" (Beier et al. 1970) or with processing of proteins in the uterine lumen (Denker 1971). Besides its unknown function, APM is now a widely used marker for apical membranes at highly polarized cells (MDCK cells, Louvard 1980; thyroid epithelial cells, Feracci et al. 1981; hepatocytes, Tanaka et al. 1986) and in this respect we have also found it useful in our study.

γ-Glutamyl transferase

GGT hydrolyzes γ-glutamyl compounds (McDonald and Barrett 1986). Its physiological substrate is thought to be

mainly the tripeptide glutathione. It is believed that GGT participates in the translocation of amino acids across cellular membranes as a consequence of its role in the γ-glutamyl cycle (Orlowski and Meister 1970; Meister and Tate 1976; Lehninger 1977). GGT showed in our experiments basically the same activity pattern as aminopeptidase *M*: main activity in the secretory phase between 5 and 6 days in pregnancy as well as pseudopregnancy and a decline in activity at 7 and 8 d p.c./p. hCG.

No main differences between implantation chamber and interblastocyst segments could be detected. In a biochemical study by Tarachand et al. (1981) on the rat uterus, GGT was found to rise in total endometrial homogenate during deciduoma formation similar to alkaline phosphatase, which is regarded as well as a marker for decidualization (Manning et al. 1969; Finn and Hinchliffe 1964).

GGT was found in the apical membrane especially of the glandular epithelium during the secretory phase in the uterus of marmoset and rat (Blanke and Graf 1985) and is discussed to be progesterone-dependent.

GGT proved in our study to be a useful polarization marker like alkaline phosphatase and aminopeptidase *M*. For the same reason it has been employed in a study by Rabito et al. (1984).

Dipeptidyl peptidase IV

In the rabbit uterine epithelium DPP IV activity was rather strong at 5 and 6 d p.c./p. hCG, declined at 7 d p.c. in interblastocyst segments and became rather weak until 8 d p.c. or 8 d p. hCG, but not yet at 7 d p. hCG. In contrast to APM and GGT the luminal epithelium of the implantation chamber retained his high activity until 8 d p.c., except for the placental fold.

DPP IV releases dipeptides, primarily the N-terminal glycylproline, from various oligopeptides and was proposed, therefore, to be possibly involved in collagen metabolism (McDonald and Schwabe 1977).

In our study it is attractive to assume a possible role of DPP IV in the adhesion of the blastocyst to the uterine epithelium since the activity remains high specifically in the implantation chamber. Indications for a possible involvement of this enzyme in cell-matrix interactions of BHK fibroblasts and rat hepatocytes were given by Hanski et al. (1985). Like GGT the activity of DPP IV was found to be rather strong in the glandular epithelium of the marmoset during the secretory phase (Blanke et al. 1984) indicating a probable progesterone-dependency of this enzyme.

Implications for the cell biological basis of endometrial receptivity

The enzymes discussed above were used in this study as cytochemical markers for the apical membrane of the uterine epithelial cells with which the trophoblast has to interact first when implantation is initiated. Epithelial cells are characterized by their polar organization which involves not only a polar arrangement of intracellular organelles and vectorial transport routes but also the existence of different cell surface domains at the apical versus the basolateral region.

The enzymes aminopeptidase *M*, γ-glutamyl transferase, alkaline phosphatase and dipeptidyl peptidase IV, which were used as markers in this study, are typically localized in the apical part of membrane compartments (see Kinne 1976; Feracci et al. 1981; Louvard 1980), whereas for example Na^+, K^+-ATPase is only localized in the basolateral membrane in well polarized epithelial cells (Kyte 1976; Kugler 1984).

The cell membrane polarity of polarized cells is maintained by tight junctions which separate the apical components from the basolateral ones (Pisam and Ripoche 1976). Besides the well documented ultrastructural differences between these two membrane domains there are also indications for biochemical differences as reviewed by Spicer et al. (1981) for glycoconjugates and by Rodriguez-Boulan et al. (1983) for different surface enzymes and ion and metabolite transporting systems.

The different reaction of the deepest crypts of the endometrium compared to middle and upper crypts in case of APM, aP and DPP IV may be explained by the existence of different cell populations in the uterine epithelium, as it was proposed by Conti et al. (1984).

In the implantation chamber, specific features have been observed for aP and DPP IV: the activity of aP raised again from 7 to 8 d p.c. and the activity of DPP IV remained high until 8 d p.c. in the cavum epithelium of the para- and oblacental folds. These phenomena may be due to steroid hormones or other mediators that may be produced by the blastocyst, although knowledge is still very limited in this field (see Dickman et al. 1976 and Kennedy 1983).

It may be argued that the general loss of enzyme activity observed here may be due to proteolytic degradation caused by the proteinase system which is known to become activated around implantation time (Denker 1977). In fact, Mancarella et al. (1981) suggested that a cell surface-bound aminopeptidase is liberated and shed into the uterine lumen by proteolytic action in the pig. However, it appears improbable that this is the major mechanism behind the observed phenomenon in the rabbit uterus, because APM depletion was still detected even after instillation of the broad spectrum proteinase inhibitor aprotinin (Trasylol®) in vivo (Denker, unpublished).

We conclude, therefore, that the observed loss of membrane enzyme activity does most probably indeed reflect a general and dramatic change in the composition of apical plasma membranes of the uterine epithelium, which occurs at a time when this tissue becomes accessible for trophoblast attachment and invasion ("receptive state"). This is in agreement with findings by Lampelo et al. (1985), who described three proteins in plasma membrane-enriched preparations of rabbit uterine epithelium which were only evident at day 6 of pregnancy (compared to day 3). They conclude that acquisition of receptivity is influenced by changes in protein composition of endometrial plasma membranes. Anderson et al. (1986) likewise described the appearance of three additional proteins in rabbit uterine epithelial plasma membrane preparations during early pregnancy, and Anderson and Hoffman (1984) noted a loss of surface negativity by day 6 of pregnancy or pseudopregnancy accompanying profound alterations in rabbit uterine epithelial glycocalix. Our study suggests, however, that changes occurring at the apical surface of the uterine epithelium do not only comprise the glycocalix but also numerous integral membrane proteins and are thus much more substantial.

Further investigations using an immunohistochemical approach should prove whether the loss of enzyme activity is due to loss of enzyme molecules or to inactivation.

Acknowledgments. We would like to thank Gerda Helm for her excellent technical assistance, Gabriele Bock for photographic work and Gisela Mathieu for typing the manuscript. These investigations were supported by Deutsche Forschungsgemeinschaft Postdoktorandenstipendium to I. Classen-Linke and by grant De 181/9-6.

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