Symplasma formation and decidualization in the pseudopregnant rabbit after intraluminal instillation of tricaprylin

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Summary. A pure neutral triglyceride, tricaprylin, was injected (Day 0) into isolated segments of the uterus of rabbits in which pseudopregnancy had been induced by i.v. injection of hCG 6 days before. The animals were killed in groups of 3 on Days 1, 2, 3, 4 and 6. Saline, simple ligation of the uterus or uterine trauma were used as control treatments in other uterine segments in the same animals. Extensive symplasma formation in the uterine epithelium of the tricaprylin-treated segments was observed on Days 2 and 3; this had degenerated by Day 4 and was absent by Day 6. Decidualization of the stroma began on Days 3 and 4 and was absent by Day 6. The symplasma formed earlier on the antimesometrial side than it did laterally or mesometrially. Decidualization was maximal mesometrially, intermediate laterally, and absent antimesometrially. Ligation with saline and trauma, but not ligation alone, produced symplasma formation. Decidualization occurred after trauma but not after saline injection or ligation alone.

Introduction

Many of the oils used as vehicles for fat-soluble materials such as the steroidal sex hormones have significant oestrogenic activity (Zarrow, Yochim & McCarthy, 1964). During a study of the endometrium of the pseudopregnant rabbit it was observed that the vehicle being used, the non-oestrogenic pure triglyceride tricaprylin, was itself effective in producing not only fusion of the endometrial epithelium (symplasma formation) but also decidualization of the stroma. In view of the potential use of this material in reproductive research we examined in more detail the effects of intrauterine instillation of tricaprylin in the 6-day-pseudopregnant rabbit.

Materials and Methods

Mature New Zealand White rabbits were isolated for several weeks and then injected intravenously with a single dose of 50 i.u. hCG (A. P. L.: Ayerst Laboratories) to induce pseudopregnancy. The animals were anaesthetized 6 days later by i.v. injection of pentobarbitone sodium (Nembutal) and the uterus was exposed. Silk thread (black, braided type B Ethicon, gauge 0) ligatures were tied at intervals along both uterine horns, creating one or two isolated segments in each horn. Tricaprylin (Sigma Chemical Co., St Louis, Missouri) or sterile saline (0.154 M-NaCl) was instilled into an isolated segment in a volume of 0.1 ml. Some segments were untreated, but in others a slit was made on the antimesometrial side of the uterus, penetrating the lumen and allowing the mucous membrane to extrude through the wound which was left open. Every animal received tricaprylin while approximately one-third were also treated by each of the above procedures. The 15 animals treated were killed in groups of 3 on Days 1, 2,

3, 4 and 6 after injection (Day 0), i.e. the 6th to 12th days of pseudopregnancy. The distended uterine segments were flushed with Bouin's fluid, immersed in fresh fixative, and processed for histological study. Paraffin-wax sections of the uterine swellings were made and stained with Harris haematoxylin and eosin. Some sections were treated with the periodic acid-Schiff stain for glycogen and mucopolysaccharides; control sections were digested with salivary amylase.

Results

Control animals in which the uteri were not ligated or were ligated without injection showed the normal evolution of the pseudopregnant endometrium (Davies & Hoffman, 1973). A proliferative phase (Days 1–3) was followed by a phase of folding and relayering of the mucosa in which the cells appeared dome-like and secretory in character (Days 4–6); this in turn was followed by fusion of the epithelial cells into multinucleate masses (Days 7–10, Pl. 1, Fig. 1), and

EXPLANATION OF PLATES

All sections were prepared after fixation in Bouin's fluid and embedding in paraffin wax and were stained with Harris's haematoxylin and eosin.

PLATE 1

Fig. 1. Endometrial surface of normal 14-day-pseudopregnant rabbit. The originally columnar epithelium is converted by fusion into multinucleated cells (m). The cytoplasm of these cells is scanty and non-acidophilic. The glands (not shown) are not transformed and remain lined by columnar secretory epithelium. $\times 200$.

Fig. 2. Antimesometrial surface of the 8-day-pseudopregnant rabbit 2 days after the intra-luminal injection of tricaprylin. The surface is covered by a voluminous symplasma (s) formed by the fusion of columnar cells into a continuous multinucleated mass (compare with Fig. 1). The cytoplasm is intensely acidophilic. The glands (g) are not involved. $\times 50$.

Fig. 3. Section of the uterus of the same rabbit as in Fig. 2 showing that the antimesometrial surface (to the right) is the seat of the symplasmic transformation while the lateral and mesometrial surface (to the left) remains covered by multinucleated cells normal for this stage (8 days) of pregnancy. The lumen is indicated (l). $\times 20$.

Fig. 4. Section through the 9-day-pseudopregnant uterus 3 days after tricaprylin injection. Degeneration (d) of the antimesometrial (am) symplasma is apparent. The mesometrial (m) epithelium shows incipient symplasma formation especially at the surface but not in the crypts. $\times 50$.

PLATE 2

Fig. 5. Decidualization of the mesometrial stroma in a 10-day-pseudopregnant rabbit 4 days after the instillation of tricaprylin into the lumen. The perivascular position of the cells (d) and their vacuolated appearance is typical of the decidual cells in normal pregnancy and they contain glycogen. The vessels (v) frequently contain many polymorphonuclear leucocytes. $\times 50$.

Fig. 6. Intense degeneration of the antimesometrial symplasma (top) in a 10-day-pseudopregnant rabbit 4 days after tricaprylin injection. The mesometrial endometrium (bottom) shows a thin surface layer of residual symplasma (s) and multinucleated cells. Decidualization is not shown in this section though extensive in other mesometrial areas. $\times 50$.

Fig. 7. Antimesometrial endometrium of a 12-day-pseudopregnant rabbit 6 days after the instillation of tricaprylin. The symplasma is largely absent except for occasional, small acidophilic masses and the surface is reclothed with a mixture of multinucleated cells (m), dark cells of columnar or multinucleate cell origin, and pale cells which are ciliated (c). The decidual areas have disappeared at this stage. $\times 200$.

Fig. 8. Uterus of 9-day-pseudopregnant rabbit 3 days after saline was instilled into the lumen. There is conspicuous symplasma formation antimesometrially (to left) but none mesometrially (to the right), and there is no decidualization. $\times 20$.

PLATE 1

(Facing p. 142)

PLATE 2

subsequently degeneration of the multinucleated cells and extensive ciliation occurred until the resumption of the oestrous state (Days 20–25). The term symplasma is used to designate the result of the transformation of the multinucleated cells into the acidophilic multinucleated cytoplasmic sheet typical of pregnancy (Amoroso, 1952). The onset of fusion, the regression of the multinucleated masses and ciliation occurred earlier in the antimesometrial areas than in the mesometrial areas.

In animals injected with tricaprylin the sequence of histological changes was the same in all 3 animals on a given day and showed a characteristic progression and regression from the 1st to the 6th day after injection. On Day 1 there was no significant difference between the experimental and control animals, both of which were typical of the 7th day of pseudopregnancy (early cell fusion). On Day 2 there was extensive symplasma formation laterally and antimesometrially (Pl. 1, Fig. 2) but not mesometrially (Pl. 1, Fig. 3). Mesometrially there was simple fusion into small multinucleate masses typical of normal pseudopregnancy (e.g. Pl. 1, Fig. 1). The symplasma occupied the surface of the endometrial folds, extending into the shallow crypts, ceasing abruptly at the junction with the glands which remained lined by a columnar epithelium. The symplasmic cytoplasm was strongly acidophilic. By Day 3 after tricaprylin instillation there was massive degeneration of the symplasma antimesometrially (Pl. 1, Fig. 4) and to a less extent laterally. Symplasma formation was apparent mesometrially at the apices of the deep endometrial folds but did not involve the crypts. At Day 4 the symplasma was grossly regressed, although at no stage was it as extensive as it was antimesometrially on Days 2 and 3, with vacuolation of the cytoplasm and extensive sloughing into the uterine lumen.

An additional feature, observed mesometrially and laterally, was decidualization of the stroma (Pl. 2, Fig. 5). The stroma was oedematous and the cells contained glycogen, as shown by PAS staining. The decidual reaction was most extensive in the two large mesometrial folds and less so in the lateral folds. An antimesometrial symplasma, never as extensive laterally and antimesometrially, also showed degeneration at Day 4, especially over swollen and decidualized mesometrial folds where it was often reduced to a thin acidophilic layer (Pl. 1, Fig. 6). By Day 6 the symplasma had disappeared in all parts of the endometrium, and a surface epithelium was restored. The disappearance of the degenerated symplasma occurred by sloughing of extensive sheets into the uterine lumen. The newly constituted surface epithelium consisted of mingled multinucleated cells typical of pseudopregnancy, degenerating remnants of the symplasma, and many areas of ciliated cells (Pl. 2, Fig. 7). The glands remained unchanged, lined by a simple columnar epithelium in which were numerous mitotic figures (2–3/high power field). The mesometrial folds were occupied by a loose stroma on Day 6, which was typical of that in the oestrous animal.

The uteri injected with saline after ligation showed symplasma formation (Pl. 2, Fig. 8) but to a lesser degree than after tricaprylin. There was no decidualization. Animals in which ligation alone was performed had uteri identical to normal uteri at this stage of pseudopregnancy. In those in which the antimesometrial was cut, there was moderate symplasma formation. Decidualization was inconspicuous apart from small perivascular areas reminiscent of early decidual formation.

Discussion

The observations following the injection of a pure neutral glyceride such as tricaprylin into the uterine lumen of pseudopregnant rabbits during the evolving stages of the endometrium emphasize, as does simple ligation or injection of saline, the essentially non-specific stimulus to symplasma formation. Symplasma, most typically observed in the rabbit, is described by Amoroso (1952) as a fusion of originally columnar cells into large, multinucleated sheets with intensely acidophilic cytoplasm. The symplasma is usually found at the implantation site and

covering areas of decidua at the margin of the placenta, as well as in areas of decidualization induced by trauma or other artificial means. The symplasma differs from the multinucleated cells normally found in pseudopregnant rabbits after about Day 7 (Davies & Hoffman, 1973); such cells are smaller, contain fewer nuclei, and never acquire the voluminous acidophilic cytoplasm with surface and other specializations typical of true symplasma. Some stimulus other than that of pseudopregnancy itself is necessary for the true symplasmic transformation of multinucleated cells. The present study shows that instillation of saline or tricaprylin or exposure of the uterine mucosa to the peritoneal fluids through a mural incision are effective, tricaprylin being by far the most effective.

While essentially non-specific in many respects, the conditions for decidualization of the stroma seem more restrictive than those for symplasma formation, but like the latter have distinct regional and temporal characteristics in their development. Hoffman, Strong, Davenport & Frolich (1977) placed silastic pellets into the uterine lumen of pseudopregnant rabbits and produced symplasmic fusion of the endometrial epithelium. Impregnation of the pellets with prostaglandins induced extensive decidualization in addition to symplasma formation; impregnation with histamine, oestradiol, or cyclic-AMP was ineffective. A common denominator for symplasma formation appears to be uterine distension; the requirements for decidualization are more complex. The appearance of the decidual cell induced by tricaprylin and the perivascular origin of the cell are as described by others in natural and artificially induced decidualization (Duval, 1889; Chipman, 1903; Loeb, 1908; Mossman, 1926; Courrier & Kiehl, 1930; Elton, 1966). The temporal and regional differences between the mesometrial, antimesometrial and lateral areas of the uterine surface following the distension of the uterus with tricaprylin reflect those observed in the normal pseudopregnant rabbit (Davies & Hoffman, 1973). In both situations the antimesometrial surface responds earlier with respect to multinucleated cell formation (pseudopregnancy) or symplasma formation (tricaprylin) and degeneration begins earlier than on the mesometrial surface; the lateral areas are intermediate in their response. Decidualization, moreover, is most marked mesometrially, less so laterally, and does not occur antimesometrially. A partial explanation of the latter phenomenon is the attenuation of the antimesometrial stroma when the uterine lumen naturally distends as in pregnancy. The slight decidualization of the endometrium after exposure to the peritoneal fluids confirms the findings of Beasley & Davenport (1970).

The fate of the symplasma, which is maximal by the 2nd day after tricaprylin injection and entirely gone by the 6th day, is degeneration, vacuolation, and sloughing into the uterine lumen. The surface is reconstituted by a complex epithelial investment containing nucleated cells, small remnants of acidophilic symplasma, and extensive areas of ciliated cells. The presence of mitotic figures in the glandular epithelium, which is not involved in symplasma formation, suggests that re-establishment of the epithelium may be accomplished by the growth of new cells from the glands. The ciliated cells, also observed in the declining stages of normal pseudopregnancy after 14 days, may serve a scavenging function. The short time course of symplasma formation, decidualization, and the resolution of both processes (by the 6th day after tricaprylin injection) may reflect the rate of absorption of the active material from the injection site.

Tricaprylin, being a chemically pure compound, should prove of practical value in reproductive research, particularly in relation to decidualogenesis, which is notoriously difficult to induce with certainty and to a substantial degree in the rabbit.

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