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Vascular Changes during Regression of the Corpus Luteum of the Guinea Pig

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Abstract

Quantitative and qualitative changes in the microvasculature of the corpus luteum of the guinea pig during cyclical luteal regression were studied by light and electron microscopy. During luteal regression, between day 12 of the oestrous cycle and day 1 of the next cycle, there was a reduction in the extent of the luteal capillary bed as measured both by 'hit' counts and by differential counts of all nucleated cells. Luteal cells made up a constant proportion of $\simeq 60\%$ of the luteal tissue area up to day 1 of the next cycle, but had increased as a proportion of all nucleated cells by that stage. Both fibroblasts and macrophages increased in number and in area occupied during luteal regression. Ultrastructural changes in capillaries during luteal regression included an increased incidence and degree of protrusion of endothelial cells into the lumina of small blood vessels, and degeneration and death of many endothelial cells. In spite of the widespread presence of endothelial cell degeneration, most small vessels retained a continuous lining of apparently viable endothelium. It is concluded that in this species there is a rapid and substantial reduction in the luteal capillary bed, which commences during the early stages of luteal regression, and a mechanism is proposed by which the capillary bed may be reduced while retaining its integrity.

Introduction

There is a sharp decline in blood flow to the corpus luteum of the guinea pig during the phase of luteal regression between day 12 of the oestrous cycle and day 1 of the following cycle (Hossain *et al.* 1979). However, although there is now strong evidence that luteal regression in this species is induced by prostaglandin $F_{2\alpha}$ released from the uterus (Horton and Poyser 1976), neither the mechanism(s) by which luteal blood flow is reduced, nor the nature of any accompanying changes in the vascular bed, are known.

In the sheep, Niswender *et al.* (1976) have reported that there is a decrease in the relative volume occupied by the luteal capillary bed during regression, and it has been shown by O'Shea *et al.* (1977) that severe degenerative changes in many luteal capillaries appear at about the time that luteal blood flow commences to fall steeply. However, no comparable data are available on any other species.

In this paper we present morphometric evidence of a reduction in the extent of the luteal capillary bed by day 16 of the oestrous cycle in the guinea pig, together with ultrastructural observations on the luteal microvasculature and its changes during luteal regression.

Materials and Methods

Animals

Adult female Dunkin–Hartley strain guinea pigs, weighing 500–700 g, were used in these studies. Timing of stages of the oestrous cycle was based on daily observation for vaginal opening, and vaginal smears were taken on days when the vagina was perforate. The first day of complete vaginal opening when the smear contained abundant cornified epithelial cells with very few nucleated epithelial cells or leucocytes was designated day 1 of the cycle. All animals studied had shown at least two consecutive oestrous cycles of duration 15–19 days immediately preceding the cycle in which luteal tissue was obtained.

Tissues

Tissues for light and electron microscopy were fixed by perfusion via the thoracic aorta under urethane anaesthesia. Specimens were perfused with either paraformaldehyde–glutaraldehyde–trinitrocresol (Ito and Karnovsky 1968) in cacodylate buffer or with 2·8% (w/v) glutaraldehyde in 0·2 M phosphate buffer, pH 7·4. Perfusion of fixative was performed without prior flushing of the vascular system, at a constant pressure of 13–14 kPa. All tissues were post-fixed in 1% (w/v) osmium tetroxide in cacodylate or phosphate buffer, stained *en bloc* in 0·5% (w/v) aqueous uranyl acetate, and embedded in Araldite. Thick sections (0·5–1·0 μm) for light microscopy were stained with 0·1% Azure II in 1% borax (Jeon 1965). Thin sections for electron microscopy were stained with uranyl acetate and lead citrate.

Tissues for the study of morphological changes in luteal capillaries during luteal regression were obtained from guinea pigs on days 9 (four animals), 12 (five), 16 (four) and 1 (five) of the oestrous cycle. On day 1, the corpora lutea of the previous cycle were studied.

Morphometric Studies

For light microscopic counts of total nuclei per unit area of luteal tissue, a calibrated eyepiece insert (the central square in a Zeiss 100 point eyepiece grid), was used to define an area of 3600 μm^2. Counts were made, at $\times 100$ magnification, from a single corpus luteum from each of three animals on each of the four days of the oestrous cycle studied. From each animal, one section was cut from each of three blocks of luteal tissue, and all nuclei except those of cells lying within the lumina of blood vessels were counted in five randomly selected areas from each section.

It has been shown previously (Hossain *et al.* 1979) that luteal weight in the guinea pig falls during luteal regression. To enable changes in weight to be converted to changes in volume, measurements of the specific gravity of luteal tissue on the same 4 days of the cycle were made on two or three freshly dissected unfixed corpora lutea from each of 15 guinea pigs (day 9—three guinea pigs; day 12—four; day 16—three; day 1—five), using flotation in a series of copper sulfate solutions of known specific gravity (Long *et al.* 1968).

'Hit' (point) counts (Weibel 1963) were performed at a magnification of $\times 1000$ on single light microscope sections from each of three blocks from each animal studied, using a Zeiss 400 point eyepiece grid. A total of 1000 hits was counted from each animal, and counts were performed from three animals on each day of the oestrous cycle studied.

Differential counts of nucleated cells, excluding cells in the lumina of blood vessels, were performed on low magnification ($\simeq \times 3000$) electron micrographs of randomly selected whole grid squares from three blocks from each of three animals on each day of the oestrous cycle studied. Only those cells whose nucleus was included in the micrograph were counted. At least 500 cells were counted on each day of the cycle. In accordance with principles outlined by Weibel (1963), numbers of nuclei of different uninucleate cell types per unit area of tissue will accurately reflect the proportions of these cells in the tissue only if their nuclei are of equal dimensions. Thus, to the extent that nuclei of different cell types in the corpus luteum of the guinea pig vary in size or shape, counts of this sort can provide only an approximate measure of relative cell numbers.

Data from all of the above counts were analysed by analysis of variance and the Student–Newman–Keuls procedure (Sokal and Rohlf 1969).

Results

Morphometric Studies

Total nuclei per unit area

Results are shown in Table 1. The number of nuclei per unit area was higher on days 16 and 1 than on days 9 and 12 ($P < 0{\cdot}01$), and was higher on day 1 than

Table 1. Number of nuclei per unit area, and luteal weight, volume and cross-sectional area on various days of the oestrous cycle

** Differences from day 9 significant at $P < 0{\cdot}01$

Parameter	n[A]	Days of the oestrous cycle			
		9	12	16	1
Mean (±s.d.) number of nuclei per unit area	3	12·4 ±1·0	13·3 ±1·0	19·0** ±1·8	22·3** ±1·6
Area occupied by 12·4 nuclei, expressed as proportion of area occupied at day 9 (%)		100	93	65	56
Mean (±s.d.) weight of a single corpus luteum (mg)[B]	5	4·07 ±0·8	3·71 ±0·4	1·91** ±0·2	1·34** ±0·1
Mean volume of a single corpus luteum (μl)		3·84	3·50	1·80	1·26
Mean cross-sectional area of a single corpus luteum (mm²)		2·45	2·31	1·48	1·17
Cross-sectional area expressed as proportion of area at day 9 (%)		100	94	60	48

[A] Number of animals per group.
[B] Data from Hossain *et al.* (1979).

Table 2. Composition of luteal tissue as determined by the 'hit' count method on various days of the oestrous cycle

Values are means for three animals. ** Differences from day 9 significant at $P < 0{\cdot}01$

Tissue components	Mean percentage composition (±s.d.) on following days of the oestrous cycle:			
	9	12	16	1
Luteal cells	59·0 ±4·1	59·0 ±1·0	60·0 ±4·0	60·0 ±2·0
Vascular tissue				
Endothelial cells and pericytes	17·5 ±1·4	16·0 ±2·2	12·0** ±1·7	11·0** ±1·8
Lumen and contents	13·0 ±2·5	11·0 ±1·4	8·0** ±1·0	7·0** ±0·2
Other components				
Cells	4·0 ±0·3	7·0 ±1·4	13·0** ±4·0	14·0** ±0·3
Non-cellular components	6·5 ±1·5	7·0 ±1·4	7·0 ±0·3	8·0 ±1·4
Total	100·0	100·0	100·0	100·0

on day 16 ($P < 0{\cdot}05$). There was no significant difference between counts on days 9 and 12. Area occupied by 12·4 nuclei (the mean number of nuclei per 3600 μm^2 on day 9) is also expressed as a percentage of the area occupied at day 9.

Table 3. Cellular composition of luteal tissue as determined by differential counts of nucleated cells on various days of the oestrous cycle

Values are means for three animals. *, ** Differences from day 9 significant at $P < 0{\cdot}05$, $P < 0{\cdot}01$ respectively

Cell types	Mean percentage cellular composition (±s.d.) on the following days of the oestrous cycle: 9	12	16	1
Luteal cells	21·9 ±2·6	22·6 ±1·6	22·3 ±3·5	29·7* ±2·2
Endothelial cells and pericytes	67·7 ±4·6	63·4 ±3·8	58·7 ±1·9	46·3** ±4·1
Fibroblasts	4·0 ±1·1	8·8* ±1·9	10·1* ±3·3	13·1** ±2·5
Macrophages	1·1 ±0·2	1·3 ±0·5	3·8** ±1·3	8·1** ±1·6
Other cells	5·3 ±2·5	3·9 ±1·9	5·1 ±3·3	2·8 ±1·0
Total	100·0	100·0	100·0	100·0

The specific gravity of luteal tissue did not change significantly between days 9 and 1 of the cycle, and the overall mean was 1·060±0·01 s.d. This mean was used as the denominator to convert weight of the corpus luteum to volume in each group (see Table 1). Cross-sectional area of the centre of the corpus luteum was then calculated assuming that the corpus luteum was a sphere, using the formula area = volume$^{2/3}$. These areas are also expressed as percentages of the area at day 9, as an indicator of the extent of shrinkage in cross sectional area during regression up to day 1 of the following cycle. As seen in Table 1, reduction in cross-sectional area calculated in this way on days 16 and 1 was greater than that needed to account for the observed increase in nuclei per unit area on the basis of cell shrinkage.

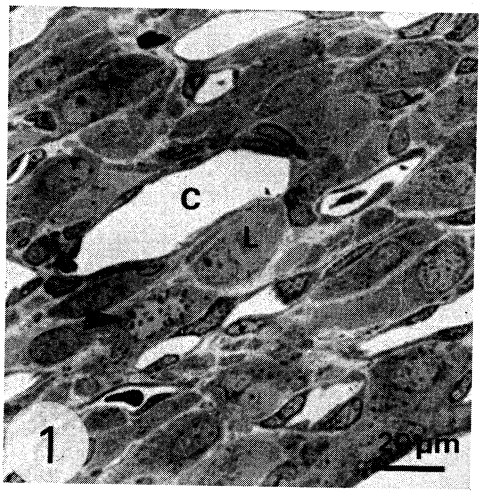
Composition of luteal tissue by 'hit' counts

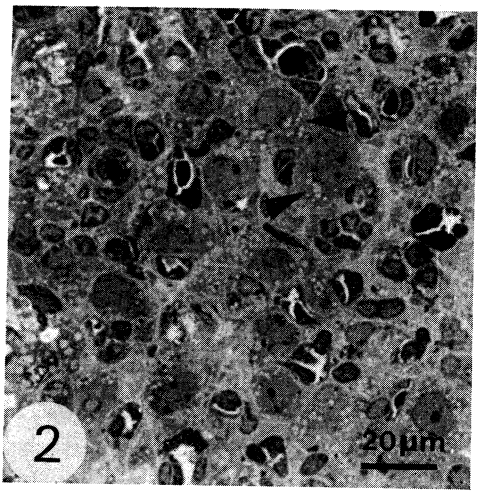
Results are shown in Table 2. As it was not always possible to distinguish endothelial cells from pericytes with certainty, these cell types have been pooled. All cells other than luteal cells, endothelial cells and pericytes were also pooled for

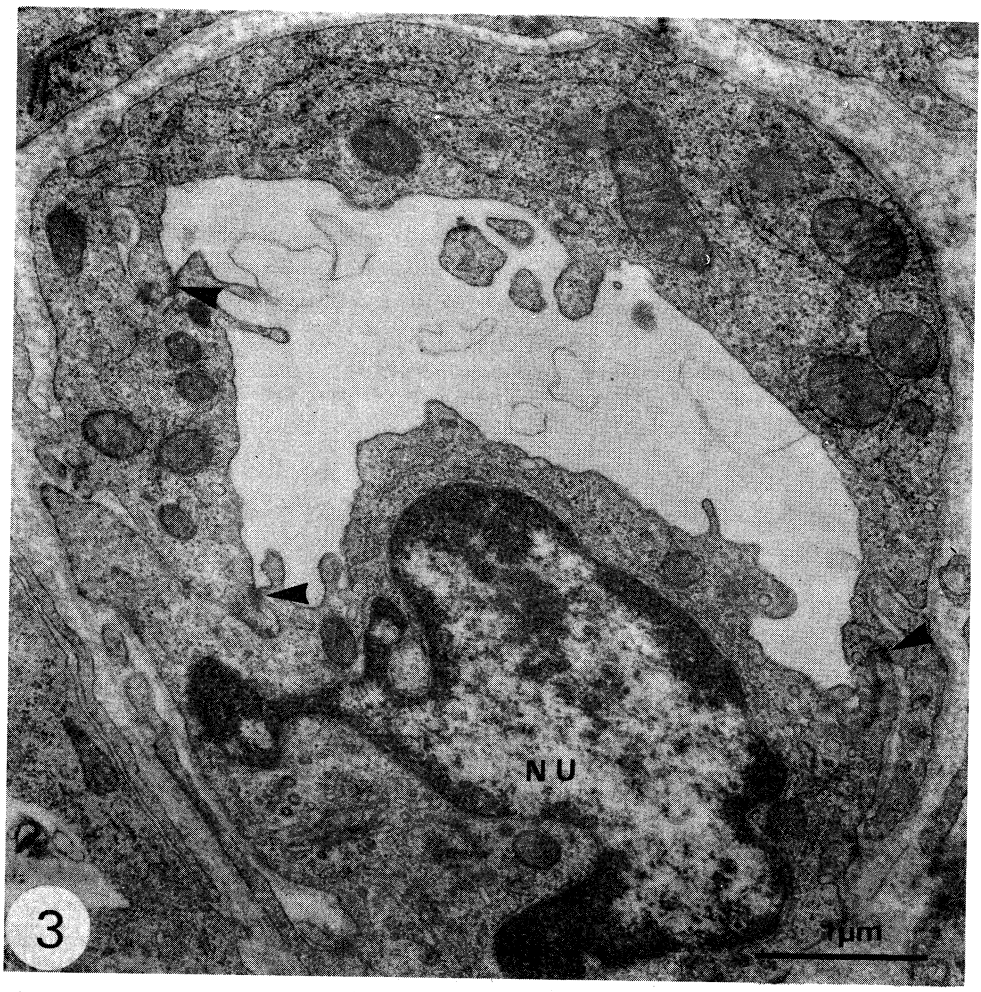
Fig. 1. Luteal tissue at day 9 of the oestrous cycle. Luteal cells (*L*) including one in mitosis (arrow), are mixed with many large and small capillaries (*C*), which are particularly prominent in this section. Azure II.

Fig. 2. Luteal tissue at day 16. The luteal cells contain numerous lipid droplets (arrows), and blood vessels are less conspicuous. Azure II.

Fig. 3. Capillary of normal appearance from a corpus luteum on day 12 of the oestrous cycle. The endothelial cell containing a nucleus (*NU*) bulges into the lumen. Interendothelial junctions are arrowed.


C
L
1
20µm


2
20µm


N U
3
1µm

counting purposes. The nature of these other cell types is considered further in the section below.

Throughout the period studied there was no change in the relative areas occupied by luteal cells or non-cellular elements. However, the proportion of hits falling on the walls and lumina of blood vessels fell sharply after day 12, being lower on days 16 and 1 than on days 9 or 12 ($P < 0{\cdot}01$). There was no significant difference between counts on days 9 and 12. In compensation for this fall there was a sharp rise in hits on cells other than luteal cells or cells of capillary walls.

Differential counts of nucleated cells

Results are presented in Table 3. From these data it can be seen that although luteal cells occupied $\simeq 60\%$ of the volume of luteal tissue (Table 2), their nuclei represented only $\simeq 20\%$ of the total observed in sections of luteal tissue in the functional phase of luteal lifespan. This proportion remained constant until day 16 of the cycle, after which there was a small rise.

The combined proportion of endothelial cell and pericyte nuclei represented around two-thirds of all nuclei observed in sections at day 9 of the cycle. Although the mean percentage of these nuclei was slightly lower on days 12 and 16 a significant fall, to less than half of the total nuclei observed, was first detected on day 1 of the following cycle.

Fibroblasts, identified by their fusiform shape, high content of rough endoplasmic reticulum and absence of a basal lamina, and macrophages, whose identification in guinea pig corpora lutea has been considered in some detail by Paavola (1979), were both observed infrequently at day 9. Both of these cell types were seen in larger numbers at day 16, and at day 1 of the following cycle. In addition to fibroblasts and macrophages, a small and apparently unchanging number of other cells, including occasional plasma cells and blood leucocytes, together with a few cells whose identity was not established with certainty, were also present.

Morphology of Luteal Capillaries

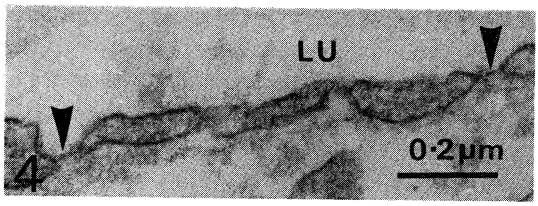
The general appearance of luteal tissue on days 9 and 16 of the oestrous cycle is shown in Figs 1 and 2. In the fully functional corpus luteum on day 9 (Fig. 1), the luteal cells were interspersed with numerous capillaries, many of which were of a large, sinusoidal type. The large and small types of capillary were frequently seen to be in direct continuity with one another, and contained occasional erythrocytes even in perfused specimens.

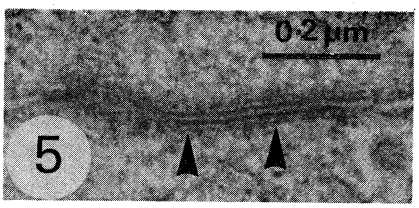
Fig. 4. Part of an endothelial cell from the wall of a capillary, showing fenestration. Diaphragms (arrows) cover the fenestrae. The lumen (*LU*) is at top.

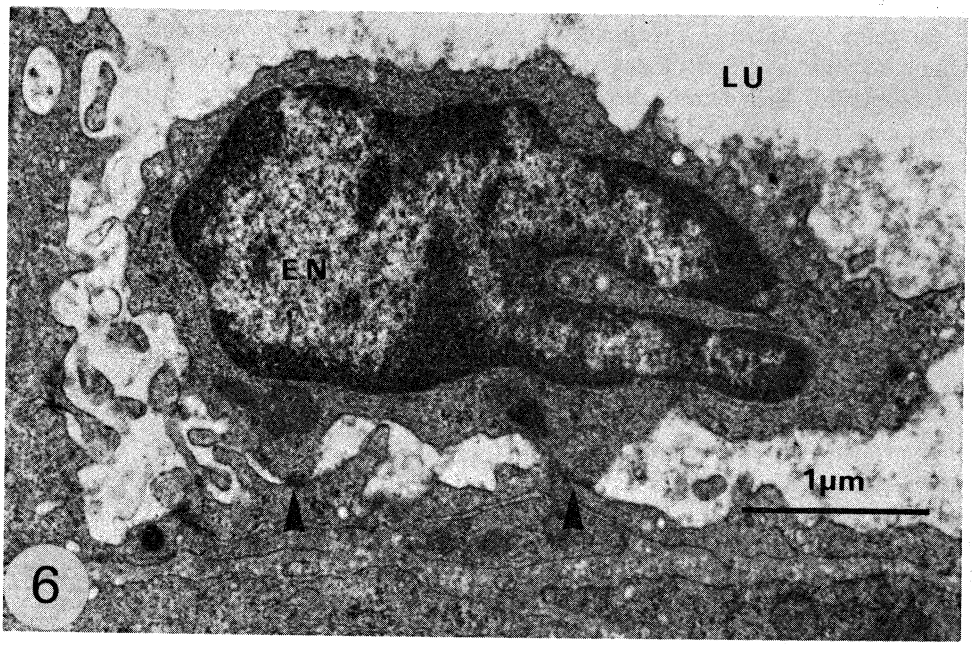
Fig. 5. Part of a capillary interendothelial cell junction, showing an adherens-type attachment (arrows).

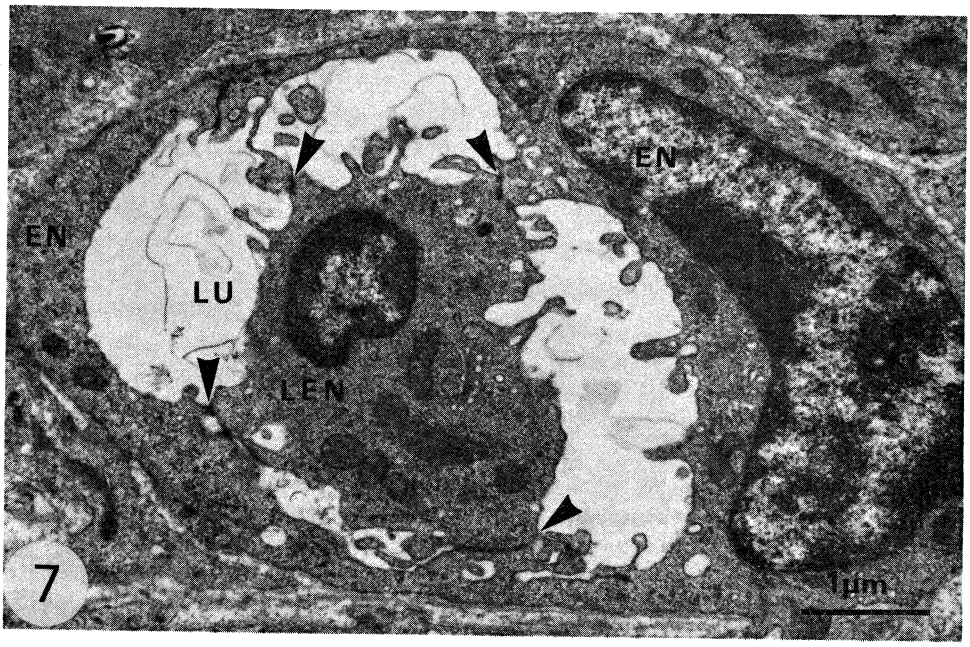
Fig. 6. From the wall of a large capillary at day 16, showing an endothelial cell (*EN*) lying within the lumen (*LU*) and attaching at two points (arrows) to the surrounding mural endothelium.

Fig. 7. Small capillary from a corpus luteum at day 16, in which one endothelial cell (*LEN*) appears to lie within the lumen (*LU*), attached only to the luminal surfaces of the surrounding endothelial cells (*EN*). Sites of attachment are arrowed.


LU
0·2μm
4


0·2μm
5


LU
EN
1μm
6


EN
EN
LU
LEN
1μm
7

The ultrastructure of large and small capillaries at day 9 of the cycle was similar. These vessels were lined by a single layer of endothelium supported by a well-developed basal lamina (Fig. 3). Pericytes formed a discontinuous layer around their walls.

The cytoplasm of the endothelial cells was generally thin, although these cells showed some tendency to bulge into the lumina of vessels at the sites of their nuclei (Fig. 3). There were no gaps in the endothelial lining, although some capillaries possessed fenestrae which were always covered by thin diaphragms (Fig. 4).

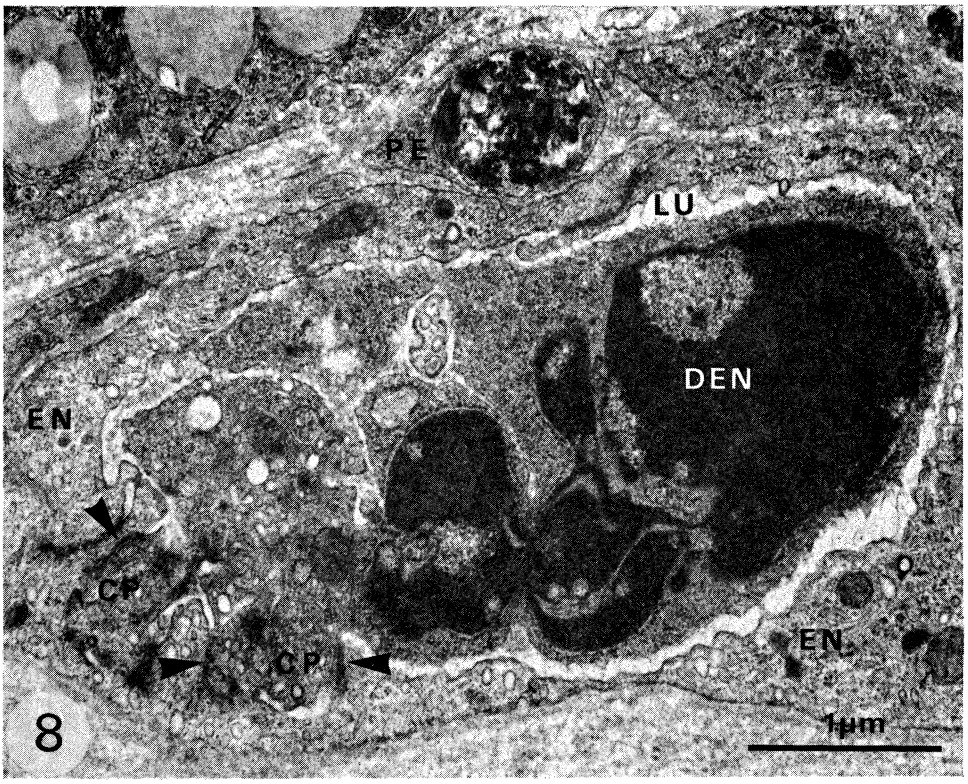
In some endothelial cell junctions the edges of adjacent cells met end-to-end almost at right angles to the lumen, while in others the margins of adjoining cells overlapped for considerable distances. Throughout the greater part of their areas of apposition, adjoining cells showed no specialization of structure and their plasma membranes simply ran in parallel, separated by a narrow space. However, localized regions of each junction, especially towards the luminal side, showed specialized forms of contact. These consistently included an adherens (intermediate) type of junction (Fig. 5), which produced characteristic foci of electron density in low magnification micrographs (Fig. 3). Small, focal occludens-type attachments were also observed towards the luminal sides of some, but not all, interendothelial junctions.

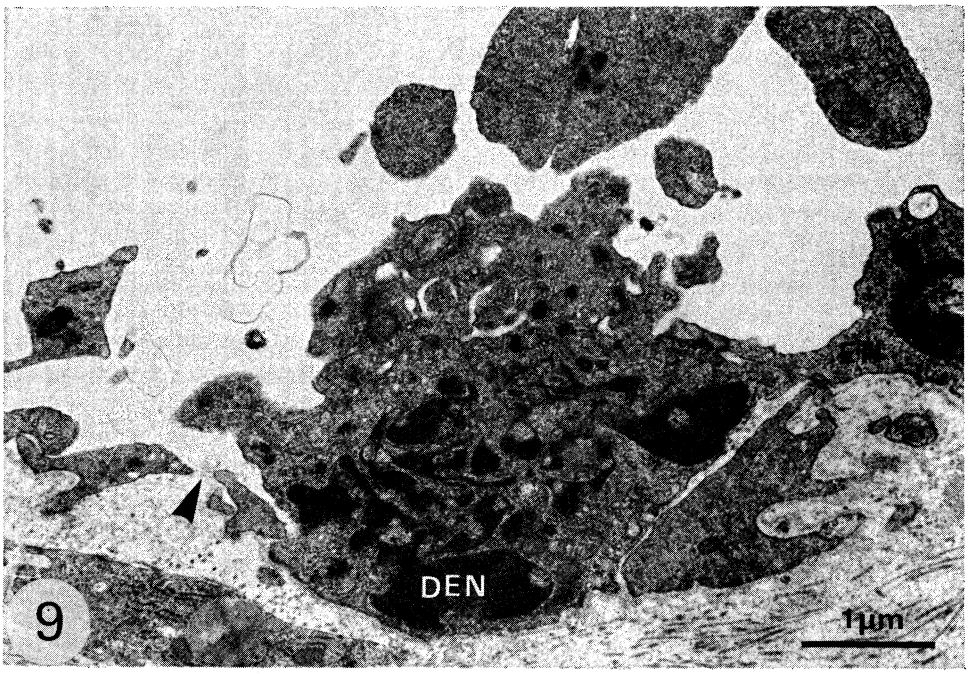
During luteal regression there was a marked reduction in the size and prominence of the capillaries (Fig. 2). Although many capillaries remained normal in appearance, others showed changes both in their walls and in their luminal contents. Changes in the walls involved both endothelial cells and pericytes, and included the appearance of cytoplasmic lipid droplets, multivesicular bodies and autophagic bodies, and a marked increase in the numbers of microfilaments and microtubules. Endothelial cells also showed an increased tendency to protrude into the lumen, resulting in extreme cases in some endothelial cells appearing to lie entirely within the lumen, anchored only to the luminal surfaces of neighbouring cells (Figs 6 and 7). Evidence that these intraluminal cells were endothelial in nature was derived from their ultrastructural features and their possession of characteristic adherens-type junctions with the surrounding mural endothelial cells.

Capillary endothelial cell death and degeneration was seen both in vessel walls and within lumina. Individual cells, distributed in an apparently random manner, were involved. Affected cells characteristically showed nuclear changes including condensation of chromatin and lobation or fragmentation of their nuclei (Figs 8 and 9), together with cytoplasmic condensation and degenerative changes in cytoplasmic organelles. Cells showing these features were commonly observed lying within the lumina of capillaries, where they could sometimes be seen to be attached to the surrounding endothelial cells (Fig. 8). In other cases no attachments to the surrounding endothelium were evident. Cells showing similar features of degeneration were also present within the walls of some capillaries (Fig. 9).

Fig. 8. Small capillary at day 16, whose lumen (*LU*) is almost filled by a degenerating endothelial cell (*DEN*) bearing two cytoplasmic processes (*CP*) which appear to be attached to the surrounding endothelium (*EN*) at the sites arrowed. Part of a pericyte (*PE*) containing a large autophagic body is present at top.

Fig. 9. Wall of a large capillary at day 1, showing a degenerating endothelial cell (*DEN*) forming part of its lining. An apparently normal endothelial cell (*EN*) is present at right, and there is a small gap (arrow) in the endothelium at left.


PE
LU
DEN
EN
CP
CP
EN
8
1μm


DEN
9
1μm

In spite of the evidence of death of substantial numbers of endothelial cells, actual discontinuities in the walls of capillaries and sinusoids were rarely seen, and most capillaries, including those containing degenerating cells, were enclosed by a continuous layer of apparently viable endothelial cells. Small discontinuities in the walls of affected capillaries were, however, occasionally present (Fig. 9).

Although a few recognizable erythrocytes and leucocytes remained present in the capillaries of regressing corpora lutea, there was no evidence to suggest that cells other than those of the endothelium contributed significantly to the degenerating cellular material present in many capillary lumina.

Discussion

The high vascularity of functional luteal tissue in the guinea pig is clearly illustrated in the present quantitative data. At day 9, almost a third of all hits were on the walls or lumina of blood vessels, while approximately two-thirds of all nuclei observed in thin sections belonged to endothelial cells or pericytes. Comparable values for the sheep are 10·8% (Nett *et al.* 1976) and 14·4% (Niswender *et al.* 1976) in hit count studies, and 50% (O'Shea *et al.* 1979) in a differential count of cell nuclei.

Several lines of evidence support the conclusion that there is a substantial reduction in the extent of the luteal capillary bed between days 12 and 1 of the oestrous cycle. During this period, there was a reduction in the area occupied by the lumina of vessels, and endothelial cells and pericytes decreased relative to other luteal elements both as a percentage of hits and as a percentage of cells whose nuclei appeared in thin sections. These changes occurred at a time when luteal weight and volume were decreasing sharply, to reach about a third of their maxima by day 1.

Reduction in luteal volume was presumably due in part to shrinkage of individual cells, as suggested by increases in the numbers of nuclei per unit area of tissue. However, cellular shrinkage seems unlikely to account for all of the loss of volume observed on days 16 and 1. In the first place, the reduction in calculated cross-sectional area was somewhat greater than that which would have been predicted from the numbers of nuclei per unit area of tissue. Secondly, some of the cells present in regressing corpora lutea (particularly macrophages) presumably represented additions to the original populations present at day 9. Some loss of cells from the corpus luteum between days 9 and 1 would be consistent with these observations.

Finally, direct confirmation of the death and disintegration of endothelial cells during luteal regression was provided by the light and electron microscopic morphological studies. Since no mitotic figures were observed in endothelium during the phase of regression, these data provided additional evidence of a net loss of endothelium.

Structurally, the capillary changes observed here were similar to those reported previously in sheep by O'Shea *et al.* (1977). However, changes in the guinea pig were somewhat less widespread, and in some respects less severe, than those in the sheep. Thus, in the guinea pig there was less evidence of endothelial discontinuity in the walls of affected capillaries, and total disintegration of capillaries was not observed. These differences may relate to the less rapid progression of luteolysis in the guinea pig, as discussed elsewhere (Hossain *et al.* 1979).

Capillaries lined by a continuous layer of apparently viable endothelium but containing attached or seemingly free degenerating cellular material, or intraluminally

situated endothelial cells showing little or no abnormality, were commonly observed during regression. This suggests that the cytoplasm of viable endothelial cells may be able to migrate below neighbouring cells which are destined to degenerate, either before or after the appearance of patent signs of cell death. The high content of microtubules and microfilaments observed in mural endothelial cells during regression would be consistent with this suggestion. The capacity of endothelial cells to migrate is well established (Sholley *et al.* 1977; Wall *et al.* 1978; Haudenschild and Schwartz 1979), and would not need to be great in this situation in which the diameter, and possibly the length, of the vessels in question is presumably reducing. Such a mechanism would provide a logical means of reducing the extent of a capillary bed to accommodate to a lower level of tissue activity, and to a reduced tissue volume, while retaining its structural and functional integrity.

As in sheep, evidence in the guinea pig supports the view that the degenerating cellular material found within the lumina of many capillaries during luteal regression is derived from the endothelium. The ability of this material to remain *in situ* presumably results in some instances from its direct attachment to the endothelium. Lack of flow along affected vessels may also contribute. The ultimate fate of this material is uncertain, but heterophagy by remaining viable endothelium appeared to contribute to its removal in the sheep (O'Shea *et al.* 1977). Macrophages, whose numbers increased sharply during regression, could also be involved, but seem unlikely to play a major role since their location was consistently extravascular, and no macrophages were observed in transit through the walls of capillaries. Some loss of cellular debris via the ovarian venous effluent is also a possibility.

Whether capillary changes similar to those observed in regressing corpora lutea occur in other organs undergoing involutionary changes is not known. However, similar features have been observed in ovarian follicles during atresia (O'Shea *et al.* 1978). It is a well-established generality that vascularity decreases in tissues whose metabolism is decreased (Guyton 1971), but the structural mechanisms involved do not appear to have been investigated in non-ovarian tissues. Capillary changes of the type reported here in luteal tissue could, however, easily pass unnoticed in any process of atrophy or involution which was gradual, and in which only small numbers of capillaries were affected at any one time.

Acknowledgments

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