LYSOSOMAL FUNCTION IN THE CORPUS LUTEUM OF THE SHEEP

J. T. DINGLE, MARY F. HAY* AND R. M. MOOR*

Strangeways Research Laboratory, Cambridge, and Agricultural Research Council Unit of Reproductive Physiology and Biochemistry, University of Cambridge

(Received 27 July 1967)

SUMMARY

A subcellular fraction containing particles showing the characteristics of lysosomes has been isolated from the corpus luteum of the sheep. Histochmical and biochemical observations have demonstrated that the lysosomes increase in size and fragility late in the oestrous cycle. Similar changes were not found in the corpora lutea of pregnant animals.

The observed increase in lysosomal fragility is one of the earliest changes associated with luteal regression, and is thought to be of functional significance in the involution of the lutein cells.

The possible modification of lysosomal function by the production of a cellspecific lytic factor from the uterus of non-pregnant sheep is discussed.

INTRODUCTION

The corpus luteum of the non-pregnant sheep undergoes rapid involutionary changes at the end of the oestrous cycle. In less than 24 hr. the lutein cells regress and the secretion of progesterone falls from the high level characteristic of dioestrus to almost undetectable values in oestrus (Edgar & Ronaldson, 1958; Deane, Hay, Moor, Rowson & Short, 1966). In the pregnant sheep, on the other hand, such luteal regression does not occur and the corpus luteum remains fully functional for almost the entire period of gestation.

The exact nature and sequence of the retrogressive changes which occur in the lutein cells of the non-pregnant sheep are not clearly understood, but they are known to occur quickly and in a highly selective manner. Since lysosomes play an important role in cellular degeneration it seemed likely that they might be involved in luteal regression. Moreover, the rapidity with which this process takes place in the sheep suggested that a study of the luteal tissue might throw light on some of the basic mechanisms underlying cellular involution.

The main object of the present study was to determine the lysosomal changes that occur in the corpus luteum of the sheep during the oestrous cycle and in early pregnancy, and to relate them to morphological and chemical changes that take place within the lutein cell.

* Postal address: Animal Research Station, 307 Huntingdon Road, Cambridge.
MATERIALS AND METHODS

Animals

The experiments were carried out on corpora lutea removed from the ovaries of 22 non-pregnant and three pregnant Welsh Mountain sheep. The occurrence of regular oestrous cycles in these animals was checked before the experiments were started.

The onset of oestrus was determined to the nearest 8 hr.; ovulation occurs 24 hr. later on day 1. The corpus luteum thus formed regresses in non-pregnant sheep late on day 15; on day 16, after the onset of the next oestrus, the corpus luteum becomes a corpus albicans. In pregnant sheep the corpus luteum remains functional almost to the end of pregnancy.

In this study corpora lutea were removed from non-pregnant sheep between days 11 and 15, and corpora albicantia were obtained on day 16. Corpora lutea were removed from pregnant sheep on the 25th day of gestation.

Surgical procedures

A strictly controlled procedure was adopted for the removal of the ovary containing the corpus luteum. Anaesthesia was induced with sodium pentobarbitone (22·5 mg./kg.) given intravenously. After incubation, and within 5 min. of induction, the sheep was placed on a fluothane-oxygen mixture. The reproductive tract was exposed through a mid-line incision and 10 min. after the injection of sodium pentobarbitone, the ovarian blood supply was clamped off and the ovary was removed quickly.

Immediately after removal of the ovary, the corpus luteum was dissected out. Half was used for histological and histochemical examination, and the remainder for estimation of lysosomal enzyme activity and nucleic acid content by biochemical methods.

Histological methods

A small piece of luteal tissue was fixed for 24 hr. in a mixture of ethanol, formalin and acetic acid (85:10:5), then dehydrated and embedded in paraffin wax. Sections, 6 μ in thickness, were stained with Delafield's haematoxylin and chromotrope 2R.

Histochemical methods

Acid phosphatase

The activity of this enzyme was followed by two methods; a rigorous time schedule was adhered to throughout both procedures.

(i) Burstone's naphtol AS phosphate method. This was carried out on slices of luteal tissue, less than 2 mm. thick, which were fixed for 24 hr. at 4° in formaldehyde (1 g. anhydrous CaCl₂, 60 ml. distilled water, 10 ml. 40% formaldehyde, pH adjusted to 7·0 with 0·1 N-NaOH, and volume made up to 100 ml. with distilled water). The specimens were then transferred to cacodylate-sucrose buffer (0·2 M-sucrose in 0·05 M-cacodylate buffer) at pH 7·2 for 8 hr. and finally to 1% gum acacia in cacodylate-sucrose buffer for a further 20 hr. A temperature of 4° was maintained. Sections cut at 8 μ in a Pearse-Slee cryostat at −25° were collected on gelatin-coated coverslips and air dried at room temperature for 30–60 min. They were then exposed
Lysosomal function in the corpus luteum of the sheep

327
to formalin vapour, rinsed and immersed in a medium containing Naphthol AS-MX phosphate as substrate and the dye Fast Red Violet LB (Burstone, 1958a, b). Reactions were carried out at room temperature for 10, 20, 45 and 90 min. The reaction mixture used for control sections contained, in addition, NaF at a concentration of 0·01 M. Finally the sections were counterstained in 1 % methyl green at pH 4·0 and mounted in Gurr's water mountant.

(ii) Bitensky's method. A histochemical assessment of lysosomal fragility is obtained by this technique (Bitensky, 1963) which is a modification of the original Gomori procedure. Pieces of tissue (not exceeding 5 mm. in any dimension) were frozen in hexane at –70° for 1–2 min. and then stored for 28 hr. on solid CO₂ in pre-cooled corked glass tubes. The tissue was transferred without thawing to the chuck of a Pearse-Slee cryostat set at –25°. Sections were cut at 8 μ with a knife that had been pre-cooled by surrounding the shaft with solid CO₂. Sections were picked up on coverslips (which had not been cooled) and were stored in the cryostat cabinet for up to 1 hr. until required. Fuller details of the procedure are given by Silcox, Poulter, Bitensky & Chayen (1965) and Chayen, Bitensky & Wells (1966).

Sections were incubated at 37° for 5, 10, 20, 30, 45 and 60 min. at pH 5·0 in a medium containing sodium β-glycerophosphate as substrate. The composition of this medium and the subsequent procedure for visualizing acid phosphatase activity have previously been described by Bitensky & Cohen (1965). NaF at a concentration of 0·01 M was added to the medium in which control sections were incubated.

Lipids

Frozen sections of unfixed or formalin-fixed tissue (handled as previously described for the demonstration of acid phosphatase activity) were cut at 8 μ and stained with Oil red O in 60 % aqueous triethyl phosphate (Gomori, 1952). Control sections were extracted with acetone before being treated with Oil red O.

Histological and histochemical preparations were assessed on coded slides by two independent observers.

Biochemical methods

On removal from the ovary the luteal tissue to be used for biochemical estimations was immediately immersed in ice-cold 0·25 M-sucrose buffered with 0·01 M-tris, pH 7·4. One quarter of the tissue was reserved for nucleic acid estimation, and the remainder used for the enzyme assays.

Assay of hydrolytic enzyme

After 45 min. in the cold tris buffer the tissue was homogenized, in a carefully standardized manner, in an Aldridge homogenizer (Dingle, Sharman & Moore, 1966) using four passes of 5 sec. duration and a tissue to fluid ratio of 1:20 (w/v). The suspension was sedimented at 600 g for 5 min. to remove nuclei and debris, and subsequently at 10,000 g for 20 min. to sediment lysosomes and mitochondria. The activities of acid protease and acid phosphatase were measured in the 600-g pellet (nuclei and debris) and in the 10,000-g pellet (the lysosomally bound enzymes) in the presence of 0·3 % (w/v) detergent, BRJ. 35 (Atlas Powder Co., Wilmington, Delaware); the enzyme activity in the supernatant (the 'free' enzyme) was assayed.
without detergent. The total specific activity of the tissue is defined as the sum of the three fractions. The protease was assayed for 30 min. at 37° with 2% haemoglobin as substrate as described by Dingle et al. (1966). Unless otherwise stated, activity is expressed as µg. tyrosine (Tyr)/hr./mg. wet wt. of tissue. Acid phosphatase was measured by the method of Torriani (1960), using p-nitrophenol phosphate (NPP) as substrate. Activity is expressed as µg. nitrophenol/hr./mg. wet wt. of tissue, unless otherwise stated. The ratio of ‘free’ activity, F (i.e. non-sedimentable at 10,000g) to the sum of ‘free’ plus ‘lysosomally bound’ (F+B) is taken as a measure of the fragility of lysosomes to the stress of homogenization.

Estimation of nucleic acids

The tissue was extracted according to the method of Schneider (1945) with ice cold 5% trichloracetic acid. After extraction with ethanol and ether, DNA was determined in the hot trichloracetic acid extraction by Burton’s (1956) modification of the Dische method. RNA was measured by reaction with orcinol (Mejbaum, 1939). Highly polymerized salmon sperm DNA containing 8-35% P and highly polymerized yeast RNA (B.D.H.), containing 8-55% P were used as standards. The DNA and RNA content of the tissue samples were expressed in terms of their phosphorus content, as µg. nucleic acid P/mg. wet wt. of tissue.

RESULTS

Histological observations

The histology of the sheep corpus luteum during the oestrous cycle has previously been described by Deane et al. (1966). In the present study, particular attention was paid to changes associated with luteal regression which begins on day 15. The most obvious early indication of the regressive process is nuclear pycnosis, followed rapidly by nuclear fragmentation. Corpora lutea were classified (Table 1) as showing no degeneration (Pl. 1, fig. 1), early degeneration (Pl. 1, fig. 2) or marked degeneration (Pl. 1, fig. 3). It is evident from Table 1 that on day 15 the corpora lutea from half the sheep examined showed signs of degeneration. For this reason the 15-day animals were divided into two groups: group A, corpora lutea without any signs of histological regression; group B, corpora lutea showing some involutionary changes.

Acid phosphatase

(i) Burstone’s naphthol AS phosphate method. Sites of acid phosphatase activity were shown in this test by discrete red granules which appeared within 5 min. of the commencement of the reaction. The granules were small from days 11 to 14 and in pregnant animals. On day 15 an increase in the size of the granules was observed, which was even more pronounced in the corpora albicantia. The intensity of the reaction, based on granule size, is shown in Table 1 on a one to three ‘+’ score, and is illustrated in Pl. 1, figs. 4–6. In the fluoride-containing controls enzyme activity was much reduced but not entirely abolished.

(ii) Bitensky’s method. With short incubation the reaction took the form of a few discrete stained particles situated in the lutein cells (Pl. 2, fig. 7). With longer incubation the stained particles became more numerous, appeared to increase in size and
Table 1. Histological, histochemical and biochemical characteristics of luteal tissue of the sheep during the oestrous cycle and in early pregnancy

<table>
<thead>
<tr>
<th>Days after oestrus and reproductive state of the sheep</th>
<th>Sheep no.</th>
<th>Histological degeneration (degen.) in luteal tissue</th>
<th>Lipid reaction with Oil red O*</th>
<th>Acid phosphatase visualized by Burstone's method†</th>
<th>Lysosomal fragility estimated by Bitensky's test (min.)‡</th>
<th>Lysosomal fragility estimated biochemically ( \left( \frac{F}{F + B} \right) \times 100% )</th>
<th>Nucleic acid concentration</th>
<th>Ratio ( \frac{\text{fRNA}}{\text{fDNA}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>11 (non-pregnant)</td>
<td>L3</td>
<td>No degen.</td>
<td>N.O.</td>
<td>+</td>
<td>N.O.</td>
<td>34</td>
<td>N.O.</td>
<td>N.O.</td>
</tr>
<tr>
<td>12 (non-pregnant)</td>
<td>L9</td>
<td>No degen.</td>
<td>N.O.</td>
<td>+</td>
<td>20</td>
<td>40</td>
<td>0.20</td>
<td>0.59</td>
</tr>
<tr>
<td>13 (non-pregnant)</td>
<td>L5</td>
<td>No degen.</td>
<td>N.O.</td>
<td>+</td>
<td>N.O.</td>
<td>39</td>
<td>0.12</td>
<td>0.59</td>
</tr>
<tr>
<td>14 (non-pregnant)</td>
<td>L11</td>
<td>No degen.</td>
<td>Tr</td>
<td>+</td>
<td>20</td>
<td>42</td>
<td>0.21</td>
<td>0.43</td>
</tr>
<tr>
<td>15: group A (non-pregnant)</td>
<td>L18</td>
<td>No degen.</td>
<td>+</td>
<td>+</td>
<td>20</td>
<td>37</td>
<td>0.10</td>
<td>0.37</td>
</tr>
<tr>
<td>16: corpora albicantia (non-pregnant)</td>
<td>L4</td>
<td>Early degen.</td>
<td>+ +</td>
<td>+</td>
<td>10</td>
<td>43</td>
<td>0.17</td>
<td>0.50</td>
</tr>
<tr>
<td>20 (non-pregnant)</td>
<td>L21</td>
<td>Early degen.</td>
<td>+</td>
<td>+</td>
<td>15</td>
<td>55</td>
<td>0.21</td>
<td>0.80</td>
</tr>
<tr>
<td>22 (non-pregnant)</td>
<td>L23</td>
<td>Marked degen.</td>
<td>+ +</td>
<td>+</td>
<td>Negative</td>
<td>57</td>
<td>0.10</td>
<td>0.33</td>
</tr>
<tr>
<td>24 (non-pregnant)</td>
<td>L12</td>
<td>Early degen.</td>
<td>+ +</td>
<td>+</td>
<td>Negative</td>
<td>58</td>
<td>0.26</td>
<td>0.66</td>
</tr>
<tr>
<td>25 (pregnant)</td>
<td>L26</td>
<td>No degen.</td>
<td>+</td>
<td>+</td>
<td>45</td>
<td>42</td>
<td>N.O.</td>
<td>N.O.</td>
</tr>
<tr>
<td>27 (pregnant)</td>
<td>L27</td>
<td>No degen.</td>
<td>+</td>
<td>Tr</td>
<td>30</td>
<td>46</td>
<td>0.15</td>
<td>0.37</td>
</tr>
<tr>
<td>29 (pregnant)</td>
<td>L19</td>
<td>No degen.</td>
<td>+</td>
<td>+</td>
<td>30</td>
<td>50</td>
<td>0.14</td>
<td>0.33</td>
</tr>
</tbody>
</table>

* Lipid: Tr, trace; + + + + +, increasing lipid content.
† Burstone's test for acid phosphatase: + + + + +, increasing intensity of reaction (Pl. 1, figs. 4–6).
‡ Bitensky's test for lysosomal fragility: time taken to give a minimal positive staining reaction.

§ Biochemical estimate of lysosomal fragility: ratio of 'free' (F) to 'free + bound' (F + B) hydrolytic enzyme activity.
N.O. = no observations.
were particularly conspicuous near the cell borders: diffuse cytoplasmic and nuclear staining progressively developed (Pl. 2, fig. 8). In contrast, the small cells interspersed among the lutein cells, and also the connective tissue surrounding the gland contained very few stained particles (Pl. 2, figs. 9, 10), indicating low lysosomal acid phosphatase activity. In the fluoride-containing controls no cells showed a particulate staining reaction.

The shortest incubation time required to produce a minimal particulate reaction varied with the physiological stage of the animal (see Table 1). From days 11 to 13 of the cycle and in the 25-day pregnant animals this was 20–30 min.; on days 14 and 15 (group A) the time was reduced to 10–15 min. In corpora lutea from sheep in group B on day 15 and in corpora albicantia there was no particulate reaction, but darkly stained angular bodies of unknown nature were observed in some animals (Pl. 2, fig. 11); these bodies were sometimes stained in the control sections also.

The absence of a particulate reaction in 15-day, group B corpora lutea and in corpora albicantia is probably due to the extreme fragility of the lysosomes at this stage. Enzyme activity may have been lost in the unfixed tissue used for the Bitensky test, but retained in the fixed tissue used for the Burstone procedure, where a strong positive reaction was obtained (Pl. 1, fig. 6).

**Lipids**

The occurrence of lipid as revealed by Oil red O largely confirmed the observations previously made with Sudan black (Deane et al. 1966). Only trace amounts of lipid were present up to day 14 of the cycle and on day 25 of pregnancy. During the period of luteal regression (day 15, group B) lipid droplets increased in size and number and became a major component of the corpus albicans (see Table 1).

**Biochemical findings**

**The presence of lysosomes in the corpus luteum**

The active (progesterone secreting) corpus luteum of the sheep shows a considerable activity of acid phosphatase and acid protease (Table 2). Most of the activity of these enzymes may be sedimented from a sucrose homogenate; approximately 80% of the total acid protease and 75% of the total acid phosphatase is sedimented at 10,000 g. The activity of these enzymes is latent unless the sedimented particles are treated with detergent or frozen and thawed repeatedly. Thus, incubation of a particulate fraction without detergent, at pH 5.0 and 37°C for 30 min. in the presence of substrate, gave 30% of the acid phosphatase activity of the same preparation in the presence of 0.3% BRIJ 35. The activity of the soluble enzyme was not affected by the detergent. These properties, together with the histochemical evidence, demonstrated that the corpus luteum contains lysosomal particles.

**The total acid hydrolytic activity of the corpus luteum**

The total specific activity of acid phosphatase and acid protease in luteal tissue was measured (Table 2). On a wet weight basis no change in acid phosphatase activity was evident, but a small rise in acid protease activity was observed towards the end of the oestrous cycle. When the results were expressed per μg. PDNA, there
Table 2. Total specific activity of lysosomal acid phosphatase and acid protease in corpora lutea and corpora albicantia of non-pregnant and pregnant sheep

(The number of sheep on which the activity is based is shown in parentheses)

<table>
<thead>
<tr>
<th>Days after oestrus</th>
<th>Type of corpus luteum</th>
<th>Acid phosphatase activity</th>
<th>Acid protease activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>µg. NPP/hr./mg. wet wt.</td>
<td>µg. NPP/hr./mg. ²DNA</td>
</tr>
<tr>
<td>11</td>
<td>Cyclic, active</td>
<td>23.1 (2)</td>
<td>104 (1)</td>
</tr>
<tr>
<td>12</td>
<td>Cyclic, active</td>
<td>24.6 (2)</td>
<td>153 (2)</td>
</tr>
<tr>
<td>13</td>
<td>Cyclic, active</td>
<td>22.1 (2)</td>
<td>177 (2)</td>
</tr>
<tr>
<td>14</td>
<td>Cyclic, active</td>
<td>21.0 (2)</td>
<td>136 (2)</td>
</tr>
<tr>
<td>15 (group A)</td>
<td>Cyclic, active</td>
<td>26.2 (4)</td>
<td>213 (3)</td>
</tr>
<tr>
<td>15 (group B)</td>
<td>Cyclic, regressing</td>
<td>27.3 (4)</td>
<td>184 (3)</td>
</tr>
<tr>
<td>16 (corpora albicantia)</td>
<td>Cyclic, regressing</td>
<td>17.1 (4)</td>
<td>175 (3)</td>
</tr>
<tr>
<td>25</td>
<td>Pregnant, active</td>
<td>20.1 (3)</td>
<td>141 (2)</td>
</tr>
</tbody>
</table>

NPP = p-nitrophenol phosphate; Tyr. = tyrosine.

Table 3. Lysosomal enzyme activity in the corpora lutea and corpora albicantia of non-pregnant sheep

(Total specific activity is the sum of the activities of (i) the low-g fraction (nuclei and debris), (ii) the high-g fraction which represents the 'bound' lysosomal enzyme activity (B) and (iii) the supernatant fraction which represents the 'free' enzyme activity (F). (Means ± s.e.).)

<table>
<thead>
<tr>
<th>Day of cycle</th>
<th>Type of corpus luteum</th>
<th>No. of animals</th>
<th>Acid protease activity, (µg. Tyr./hr./mg. wet wt.)</th>
<th>Acid phosphatase activity, (µg. NPP/hr./mg. wet wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td>Free</td>
</tr>
<tr>
<td>11 day–15 day (group A)</td>
<td>Active</td>
<td>12</td>
<td>11.8 ± 0.5</td>
<td>2.6 ± 0.2</td>
</tr>
<tr>
<td>15 day (group B) and 16 day (corpora albicantia)</td>
<td>Regressing</td>
<td>8</td>
<td>13.9 ± 2.8</td>
<td>5.7 ± 0.6</td>
</tr>
</tbody>
</table>

P, significance of difference between active and regressing corpora lutea

|                          |                       |                | 0.5     | < 0.001 | < 0.001 | 0.6     | < 0.001 | < 0.001 |

NPP = p-nitrophenol phosphate; Tyr. = tyrosine.
was a slight rise in both acid phosphatase and acid protease activity. However, this rise is of doubtful significance since no statistically significant difference could be found (see Table 3) between the total specific enzyme activity in actively secreting cyclic corpora lutea (day 11 to day 15, group A) and those showing histological signs of regression (day 15, group B and corpora albicantia). Moreover, the total enzyme activity of actively secreting corpora lutea of pregnancy was of the same order as that of the regressing luteal tissue.

**Lysosomal fragility**

The ratio F:F + B can be taken as a measure of fragility of lysosomal particles to the stress of homogenization. Alteration in the ratio, which has been shown to change under various pathological conditions (Dingle & Lucy, 1965), may be due to such factors as difference in particle size, membrane stability, enzyme activity or physiological function of the lysosome population at the time of isolation. This ratio (F:F + B) with acid protease and acid phosphatase as the marker enzymes (Text-fig. 1) is relatively constant between day 11 and day 15, group A, but rises steeply during luteal regression (day 15, group B and day 16). The highly significant difference in this ratio between actively secreting luteal tissue (day 11 to day 15, group A) and the regressing glands (day 15, group B and day 16) is shown in Table 3. The mean ratios (F:F + B) in the actively secreting tissue are 36.8 ± 0.6 and 43.5 ± 2.0 for the protease and phosphatase respectively; the comparable ratios in the regressing glands are 61.7 ± 0.8 and 66.8 ± 3.1.

Lysosomes from the corpora lutea of pregnant animals did not show any such increase in fragility to the stress of homogenization (Table 1 and Text-fig. 1).
Correlation of histochemical and biochemical results

The histochemical fragility test of Bitensky (1963) as used in this study gave results similar to the biochemical assay of lysosomal fragility (Table 1). Thus staining times of 20-30 min. were associated with mean ratios of F:F+B (using combined acid phosphatase and acid protease values for each tissue) of 47 or less, but ratios of F:F+B of 50 or more were correlated with either reduced staining time, or completely negative results, indicating highly unstable (fragile) lysosomes.

Nucleic acid content

The concentration of nucleic acid in the luteal tissue is given in Table 1. The mean PDNA:wet wt. ratio was slightly higher in the regressing corpora lutea (0-19) than in fully active glands from the non-pregnant sheep (0-15). This difference could be accounted for by the loss of cellular material during regression, since the mean PRNA:PDNA ratio in the regressing corpora lutea (2-7) was slightly lower than that for active corpora lutea (3-3). However, the significance of this result is doubtful in view of the large variation found in the regressing tissue (Table 1).

DISCUSSION

A subcellular fraction containing particles with the characteristics of lysosomes (De Duve, 1959) has been isolated from the corpus luteum at different stages of the oestrous cycle. The biochemical studies, together with the histochemical observations, demonstrate that the lutein cells are richly endowed with lysosomes. These particles undergo marked changes in functional activity in late dioestrus. The increase in lysosomal activity is one of the earliest changes associated with luteal regression and is thought to play an important part in the involution of the corpus luteum of the non-pregnant sheep.

The sites of acid phosphatase staining observed in the histochemical preparations may correspond to the electron-dense particles of 0·3-0·5 μ diameter previously seen in electron micrographs of lutein cells of the sheep (Deane et al. 1966). The apparent increase in the size of the lysosomal particles on day 15-16 may be related to the formation of autophagic vacuoles. In the light of the present study, the electron micrographs of Deane et al. (1966), together with recent unpublished material of the same authors, were further examined; in the micrographs of regressing corpora lutea numerous autophagic vacuoles were seen. The presence of an increased number of these vacuoles might at least partially explain the increased fragility of the lysosomal population after day 15.

A striking feature of luteal regression in the non-pregnant sheep is the rapidity with which it occurs: on day 15 the synthesis and secretion of progesterone falls in less than 24 hr. from a maximum level to an almost undetectable one (Edgar & Ronaldson, 1958; Short, 1964). This drop in secretory activity is accompanied by cytological and histochemical changes which are initially localized in the lutein cells themselves (Pl. 2, figs. 9, 10). The increase in the fragility of the lysosomes, observed on day 15, may well be associated with both the rapidity and the specificity of luteal regression.
In the rat, lysosomes have been shown histochemically to be present in the lutein cells and also in associated macrophages, and it has been suggested that these lysosomes may play a part in luteal regression (Novikoff, 1960; Lobel, Rosenbaum & Deane, 1961). However, in the rat, in contrast to the sheep, it appears that much of the lysosomal enzyme activity is localized in the invading macrophages, rather than in the lutein cells themselves (Banon, Brandes & Frost, 1964).

It has been established in the sheep that involution of the corpus luteum at the end of the oestrous cycle is dependent upon the presence of the non-pregnant uterus (Wiltbank & Casida, 1956; Denamur & Mauleon, 1963; Moor & Rowson, 1964). It has, moreover, been found that the corpus luteum is affected more markedly by the lytic influence of the adjacent uterine horn than by that of the contra-lateral uterine horn (Moor & Rowson, 1966). The mechanism by which the uterus affects the corpus luteum is not yet known, but the lysosomes of the lutein cells are ultimately involved. It is possible that the lytic influence from the uterus directly affects the lysosomes; the resultant increase in their fragility may be the primary cause of luteal regression. Alternatively, some other element of the lutein cell may initially be affected, the changes in lysosomal function thus being of a secondary though nevertheless important nature. An analogous situation may be the effect of complement-sufficient antiserum which, though it causes changes in lysosomal activity, has no direct action on the lysosomes but is thought to act indirectly by way of changes in the plasma membrane of the cell (Dingle, Fell & Coombs, 1967). This concept might provide a mechanism for antibody involvement in luteal regression. Another possible indirect yet cell-specific mechanism involving lysosomes could be related to the inhibition of progesterone synthesis or secretion. Thus, cessation of steroid release might lead to autophagic changes in the cell in a manner somewhat similar to that seen in the mammotrophic hormone-producing cell of the lactating rat after removal of suckling young (Smith & Farquhar, 1966).

The authors are grateful to Dr L. Bitensky for advice on histochemical methods for assessing lysosomal fragility, and to Mr L. E. A. Rowson for undertaking some of the surgery. Invaluable technical assistance was rendered by Miss Bridget Bunting and Mrs Pat Miles. We thank Dame Honor Fell, F.R.S., and Professor T. R. R. Mann, F.R.S., for reading and criticizing the manuscript.

REFERENCES
Lysosomal function in the corpus luteum of the sheep


DESCRIPTION OF PLATES

**PLATE 1**
Figs. 1-3. Paraffin sections of ovine corpora lutea and corpora albicantia stained with Delafield's haematoxylin and chromotrope 2R. (x 512.)

Fig. 1. Day 12, non-pregnant (L11). An active corpus luteum showing no evidence of cellular degeneration. Between the large lutein cells are small connective tissue cells.

Fig. 2. Day 15, group B, non-pregnant (L4). Early degeneration. Shrunken cells with fragmenting nuclei (†) are interspersed amongst plump lutein cells.

Fig. 3. Day 16, corpus albicans (L23). Marked degeneration. Most of the lutein cells are shrunken, the cytoplasm is vacuolated and the nuclei are pyecotic or fragmenting. More intercellular collagen is present than in Figs. 1 or 2.

Figs. 4-6. Frozen sections of formalin-fixed corpora lutea stained for 45 min. by Burstone's naphthol AS phosphate method to show sites of acid phosphatase activity. The intensity of the reaction was scored +, ++ or +++ on the basis of granule size. (x 820.)

Fig. 4. Day 12, non-pregnant (L11). Reaction ++. Small stained particles are evenly distributed throughout the luteal tissue.

Fig. 5. Day 15, group B, non-pregnant (L12). Reaction ++. Small aggregations of stained particles can be seen.

Fig. 6. Day 16, corpus albicans (L23) Reaction ++. Sites of acid phosphatase activity are not evenly distributed and quite large aggregations of stained particles are present.
Plate 2

Figs. 7–11. Frozen sections of unfixed corpora lutea stained by Bitensky's method to show lysosomal fragility and acid phosphatase activity.

Fig. 7. Day 15, group A, non-pregnant (L18). Incubated 20 min. Discrete stained particles in the lutein cells indicate sites of acid phosphatase activity. The area shown is a little more heavily stained than was routinely accepted as a minimal positive reaction. (× 880.)

Fig. 8. Day 15, group A, non-pregnant (L21). Incubated 45 min. With prolonged incubation, the nuclei are stained, and the cytoplasmic particles become darker, particularly round the periphery of the cells; there is also slight diffuse cytoplasmic staining. (× 1714.)

Fig. 9. Day 12, non-pregnant (L11). Incubated 45 min. Numerous sites of acid phosphatase activity can be seen in the large lutein cells, but they are much fewer in the surrounding small connective tissue cells. (× 880.)

Fig. 10. Day 25, pregnant (L26). Incubated 45 min. The cells of the connective tissue capsule which surrounds the corpus luteum contain few sites of acid phosphatase activity, while numerous sites of enzyme activity can be seen in the adjacent luteal tissue. (× 880.)

Fig. 11. Oestrous (L15). Incubated 20 min. There is no particulate reaction in this section but darkly stained angular bodies can be seen. (× 880.)