CELL DYNAMICS OF THE OVARIAN CYCLE

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Summary. During the ovarian cycle the mouse ovary undergoes changes which involve considerable movements of cells. Autoradiographs prepared at different time intervals after flash labelling with \(^{3}H\)-thymidine show that it is possible to mark cells in distinct cell groups in the ovary and to follow their growth, movement and disappearance from cycle to cycle for a considerable period of time. This method has been used to follow follicle development, the movements of corpora lutea and the development of the peripheral stroma through five cycles.

INTRODUCTION

During the ovarian cycle of the mouse the ovary undergoes changes which involve considerable movements of cells. Small follicles grow into large ones, these rupture, interrupting the continuity of the surface epithelium, which closes again shortly afterwards. Corpora lutea form and are soon displaced from the periphery towards the centre of the organ, from where they ultimately disappear, as fresh material pushes inwards. Histological investigations allow just a glimpse, like a still out of a moving picture, into the happenings of the constantly changing organ. In order to visualize this dramatic process an attempt was made to mark cells of the different ovarian structures with a radioactive marker and to follow their movements by localizing the marked structures after different time intervals in autoradiographs. Cell migration in the female reproductive tract has been followed autoradiographically by Perrotta (1962) in the vaginal and uterine epithelium of the mouse and by Peckham, Barash, Emlen, Kiehhofer & Ladinsky (1963) and Peckham, Ladinsky & Kiehhofer (1963) in the vaginal epithelium of the rat.

This communication reports on the movements of cells in the ovary of mice which have been flash labelled with tritiated thymidine at the time of oestrus. To note which cells and cell structures label in the ovary at oestrus the first autoradiographs were prepared an hour after flash labelling. The fate of the cell groups which label at oestrus were then followed in autoradiographs examined one, two, three, four and five cycles later, when the mouse was again in oestrus. By this method we hoped to gain insight into some questions of growth and disappearance of certain structures in the ovary: how long it takes for a follicle to grow, how long a corpus luteum remains recognizable in the ovary, and what the history of the peripheral stroma is.

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MATERIALS AND METHOD

Twenty-two Street mice, 3 and 4 months old, were used in these investigations. In all cases daily vaginal smears were taken for about 1 month before the investigations were begun, to establish the vaginal cycles of the individual mouse. Smears were continued throughout the experiment. Only mice with regular cycles were used. At the time of oestrus the mice were injected intraperitoneally with $^3$H-thymidine (20 $\mu$C in the 1 hr experiment, 100 $\mu$C in all other cases).

Autoradiographs of ovaries were prepared for six groups of mice:

1. Injected in oestrus (E₀), killed 1 hr later.
2. Injected in oestrus (E₀), killed in oestrus one cycle later (E₁).
3. Injected in oestrus (E₀), killed in oestrus two cycles later (E₂).
4. Injected in oestrus (E₀), killed in oestrus three cycles later (E₃).
5. Injected in oestrus (E₀), killed in oestrus four cycles later (E₄).
6. Injected in oestrus (E₀), killed in oestrus five cycles later (E₅).

K₂ liquid emulsion (Ilford) was used to prepare the autoradiographs which were exposed for 30 or 60 days.

The radioactive thymidine injected during oestrus is incorporated into all those cell nuclei which at the time of injection synthesize DNA. The radioactive DNA remains in the nucleus until the cell divides, in which case each of the daughter cells will carry half of the original label, or until the cell degenerates and disappears. Any cell that remains undivided, as well as those which have divided a few times, will, therefore, remain recognizable in autoradiographs for considerable periods of time.

Several possible fates can overtake the cells that incorporated the label at the time of injection: (1) The cells did not divide between E₀ and E₁ therefore the nuclei kept the label: heavily labelled cells will be seen; (2) the cells divided between E₀ and E₁: these cells will give an autoradiographic picture of less heavily labelled cells; (3) the labelled cells remained physically in the same location as they were in E₀; and (4) the cells wandered or were displaced between E₀ and E₁.

The histological preparations were made from ovaries after fixation in Bouin’s solution. The organs were serially sectioned at 5 $\mu$ and stained either with the Feulgen reaction before or with haematoxylin and eosin after the preparation of the autoradiographs.

The following classification of the stages of the follicles were used (Peters & Levy, 1964):

Type 2: small oocyte with a few follicle cells.
Type 3a: small oocyte with a complete ring of follicle cells, usually flat.
Type 3b: growing oocyte with a complete ring of round follicle cells.
Type 4: growing oocyte with two rows of follicle cells.
Type 5: large oocyte with many rows of follicle cells.
Type 6: large oocyte with many rows of follicle cells and beginning antrum formation.
Type 7: large oocyte with many rows of follicle cells; antrum developed; corona formed.

RESULTS

Injected in oestrus ($E_0$) killed 1 hr later

Five mice were injected with $^3$H-thymidine and killed an hour later, in order to ascertain which cells in the ovary incorporate the marker at the time of oestrus. It was not clear whether these would be individual cells at random or whether whole cell groups could be marked in this way, while others could be identified because they do not incorporate the label selectively.

In four of the mice examined in this group the label was injected after ovulation had taken place. In these specimens eggs were found in the tube, which were still in close contact with accompanying follicle cells. Freshly ruptured follicles or very recently formed corpora lutea occurred in the periphery of the ovary next to large follicles some of which were degenerating. In one case the marker was injected before ovulation had taken place (the tube was not dilated and no eggs were found in the tube). The periphery of this ovary was filled with many large follicles. No fresh corpora lutea were present.

The ovaries of the animals killed an hour after injection of the label showed labelling of the nuclei of cells of various structures.

Cells of the surface epithelium, often neighbouring cells, are frequently labelled, especially in areas where this tissue covers large or recently ruptured follicles. Most characteristic is the labelling pattern of the large follicles (Types 5 to 7).

Many cells of almost all the large follicles (except those in advanced degeneration) incorporate the label (Pl. 1, Fig. 1).

In Type 4 follicles fewer cells incorporate the label (Pl. 1, Fig. 2), but there are only few of this type that have none of the follicle cells marked. However, the majority of the small follicles (Types 2 and 3a) remain unlabelled. Only 10% of their number incorporate the marker usually into a single follicle cell.

Follicle cells in recently ruptured follicles do not usually incorporate the label, and the invading theca cells remain on the whole unlabelled. Only an occasional single cell incorporates the label. However, in fresh corpora lutea, in which the gap is still distinguishable, cells which line the gap and those on the surface of the central cavity often become labelled (Pl. 1, Fig. 3). These cells seem to be similar to those which lie close to the superficial capillaries surrounding the corpus luteum, many of which incorporate the label. This gives a characteristic picture of labelled cells lying close to the surface of the fresh corpus luteum. Corpora lutea of the previous cycle ($E_0 - 1$), which are often already displaced from the periphery, remain entirely unlabelled. Their unlabelled bodies are in clear contrast to the heavily labelled large follicular structures in the periphery of the organ (Pl. 1, Fig. 2).

Cells of the stroma, peripheral as well as central, do not incorporate the label. Only some single cells in close proximity to the vessels become labelled.

In summary: tritiated thymidine injected at oestrus is mainly incorporated into many cells of the large follicles and into cells close to the peripheral capillaries. It is not incorporated into the peripheral and central stroma nor into old corpora lutea.
Ovaries injected in oestrus \( (E_0) \) and killed in the following oestrus \( (E_1) \). \( (E_0 \cdots E_1) \)

Five specimens were available for study in this group. All five ovaries had ovulated at the time of killing. Three specimens showed eggs in the tube still in close contact with follicular cells and two specimens had naked eggs in portions of the tube close to the uterus.

Ovaries marked with \(^3\)H-thymidine and examined as autoradiographs in the next oestrus presented a very characteristic appearance. Even under low power survey the attention of the observer was drawn to the recently ruptured follicles and young corpora lutea lying in the periphery of the organ (Pl. 1, Fig. 4). A rim of heavily labelled cells outlining the periphery was characteristic for the young corpus luteum formed in \( E_1 \). Closer examination revealed that the young corpus luteum consisted of two types of cells: (1) the heavily labelled ones at the periphery (which later on invade), and (2) lightly labelled ones making up the main part of the structure (Pl. 1, Fig. 5). These young corpora lutea of \( E_1 \) were flash labelled in \( E_0 \), when they were large follicles (Type 7). In the interval between \( E_0 \) and \( E_1 \) the follicle cells divided one or more times, but the theca cells did not, resulting in the distinct autoradiographic picture of the young corpus luteum 5 days after labelling. If the preparation of the autoradiograph was delayed until metoestrus, i.e. when the corpus luteum was about 3 days old, the heavily labelled cells left the periphery and were invading its body. They were lying distributed throughout the corpus luteum (Pl. 1, Fig. 6), intermixed with the lighter labelled cells. The corpus luteum of \( E_1 \) was physically in about the same position as the large follicle one oestrus before, both structures lay in the periphery. The corpora lutea of the previous cycle \( (E_0) \) did not show any labelled luteal cells. They were fresh corpora lutea at the time of \(^3\)H-thymidine injection. A few single labelled cells were distributed throughout their bodies, corresponding to the cells which in \( E_0 \) were lining the central cavity and the capillaries of the then fresh corpus luteum.

The corpus luteum of two cycles earlier \( (E_0 - 1) \), which was usually displaced from the periphery, had no labelled cells.

Few (about 8\%) of the small follicles (Type 2 or 3a) had labelled cells. An occasional Type 3a follicle was found with one or two heavily labelled cells in its ring. These are apparently follicles which did not change in size, they did not increase the number of their follicle cells between \( E_0 \) and \( E_1 \), but remained ‘inactive’ and unchanged in the periphery.

Some of the growing follicles were labelled (Pl. 2, Fig. 7). In the ring of cells surrounding the oocyte a few were often heavily labelled, whereas many of the others were only lightly labelled and some showed no label at all. It is likely that such a follicle grew in the preceding 4 or 5 days from a small follicle (Type 3a) to the present size.

Many of the large follicles (Types 5 to 7) had lightly labelled cells, suggesting that their follicle cells divided actively in the intervening time between \( E_0 \) and \( E_1 \).

The peripheral and central stroma was not labelled.

The cells surrounding the egg in the tube were lightly labelled, indicating that they incorporated the label at \( E_0 \) and divided actively in the 4 days preceding ovulation.
Fig. 1. Autoradiograph of an ovary prepared 1 hr after flash labelling in oestrus. Many cells of the large follicles incorporate the label. × 100.

Fig. 2. Autoradiograph prepared 1 hr after flash labelling. A few cells of a growing follicle are labelled. Many cells of the large follicle are labelled. The corpus luteum (c.l.) of the previous cycle is not labelled. Cells of the peripheral stroma (p.s.) and the central stroma (c.s.) do not incorporate the label. × 100.

Fig. 3. A freshly ruptured follicle in an autoradiograph prepared 1 hr after flash labelling. Very few follicle cells incorporate the label, but some cells along the capillaries and many that are lining the gap of the rupture become labelled. × 265.

Fig. 4. A fresh corpus luteum in an autoradiograph prepared in oestrus (E₁), 4 days after flash labelling in the previous oestrus (E₀). A rim of heavily labelled cells surrounds the corpus luteum. × 100.

Fig. 5. Higher magnification of Fig. 4 shows the young corpus luteum to contain heavily as well as lightly labelled cells. × 400.

Fig. 6. Ovary flash labelled in oestrus (E₀), autoradiograph prepared one cycle later in metoestrus (M₁). This corpus luteum is about 3 days old. The heavily labelled cells have left the periphery and have invaded the body of the corpus luteum. × 100.

(Facing p. 230)
Fig. 7. Ovary flash labelled in oestrus (E₀), autoradiograph prepared in the following oestrus (E₁). Growing follicle. Two cells in the follicle cell ring are heavily labelled, many of the others are lightly labelled, indicating that they divided between E₀ and E₁. The follicle ring increased its cell number, i.e. the follicle grew between flash labelling and time of killing. ×400.

Fig. 8. Ovary flash labelled in oestrus (E₀), autoradiograph prepared 2 cycles later (E₂). Contracted follicle in the periphery of the ovary with heavily labelled follicle cells. These cells incorporated the label in E₀, but did not divide. ×100.

Fig. 9. Stroma in the periphery of an ovary flash labelled three cycles before the autoradiograph was prepared. The labelled cells are part of a contracted follicle, in which a zona pellucida rest (z.p.) is still recognizable. ×400.

Fig. 10. Typical appearance of the peripheral stroma in an autoradiograph prepared three cycles after flash labelling. ×400.

Fig. 11. Growing follicle with labelled cells four cycles after flash labelling. Many heavily and lightly labelled cells are seen. ×400.
Ovaries injected in oestrus (E₀) and killed two cycles later (E₂). \((E₀ \cdots E₁ \cdots E₂)\)

The time that elapsed between the oestrus of injection and the oestrus at the time of death, two cycles later, was 8 to 10 days.

The corpora lutea. There are now three sets of corpora lutea to be considered (Text-fig. 1): (1) The corpus luteum of E₂, formed by the follicle that ruptured shortly before death; (2) The corpus luteum of E₁, i.e. the one that at the time of injection was still a large follicle; and (3) the corpus luteum of E₀, which had formed just before the label was injected.

(1) The corpus luteum of E₂ is found in the outer periphery. It has clearly two types of cells. Many nuclei are rather lightly labelled indicating that they divided many times between the time of flash-labelling and killing. The nuclei of others are more heavily labelled, they did not divide as often in the same time interval. These correspond to the theca cells. One of the specimens was obtained somewhat later than the first one (it had naked eggs in the lower part of the tube). The fresh corpora lutea of this specimen showed the lightly and heavier labelled cells well intermixed, suggesting the complete invasion of the theca cells.

(2) The corpus luteum of E₁, i.e. the one formed during the previous oestrus, can be distinguished from the corpus luteum of E₂ by two facts: first, its lightly labelled cells have more grains over their nuclei than the comparable cells of the corpus luteum of E₂. This is caused by the fact that the cells of the corpus luteum of E₂ divided as follicle cells for 8 to 10 days, diluting the label of their nuclei considerably, before this corpus luteum was formed and the follicle cells stopped dividing. The cells in the corpus luteum of E₁, however, divided as follicle cells only between E₀ and E₁ (4 to 5 days), when this corpus luteum was formed and the follicle cells stopped dividing. Their cells appeared therefore in the autoradiographs more heavily labelled than the comparable cells of the corpus luteum of E₂.

The second fact that distinguishes the corpus luteum of E₁ is that it usually does not lie in the periphery of the ovary any more but has already begun to be displaced towards the centre of the organ.

(3) The corpus luteum of E₀, which had formed shortly before the label was injected was found even more centrally displaced. It lay together with corpora lutea of earlier cycles (E₀−1) in the centre of the organ. These corpora lutea had shrunk in size and were considerably smaller than the younger corpora lutea. The corpus luteum of E₀ could still be distinguished from older ones, as it had an occasional labelled cell in its body (see above); the older ones had none.

The follicles. All large follicles (Types 6 and 7) had most of their cells lightly labelled, indicating that they probably grew steadily between the time of injection and killing. This refers to follicle as well as theca cells. Some of the Type 4 follicles, however, are characterized by some heavily labelled follicle cells which reside next to lighter labelled nuclei, suggesting that not all cells that incorporated the label 10 days before divided, indicating that the growth of these follicles was not as steady and perhaps not as rapid as that of the large ones.

The peripheral stroma. The follicles in the periphery are separated from one
another by a rather cellular stroma. Part of it consists of contracted follicles, some of which still have a central cavity in which a zona pellucida rest is recognizable, while others have been pushed together and appear as strands, their central cavity being no longer recognizable. Some of this peripheral material contains heavily labelled cells (Pl. 2, Fig. 8). It is likely that the structure seen in Pl. 2, Fig. 8 was a Type 5 follicle in E₀ at the time of flash labelling. Many of its cells incorporated the label. This follicle, however, did not develop any further, nor did its cells divide again. In the cycle that followed the oocyte degenerated. At the time of death, in E₂, the contracted follicle was still in the periphery, its cells (undivided since E₀) were still heavily labelled and began to make up the peripheral stroma.

**Ovaries injected in oestrus (E₀) and killed three cycles later (E₃). (E₀⋅⋅⋅⋅E₁⋅⋅⋅⋅E₂⋅⋅⋅⋅E₃)**

The interval between injection and killing was 14 to 15 days. Autoradiographs of four animals were examined in this group.

The overall labelling became less intense and the dilution of label more marked as the interval between injection and the preparation of the autoradiographs lengthened.

Four sets of corpora lutea had developed between the cycle of injection (E₀) and E₃. The cells of the most recent corpora lutea were only lightly labelled. These bodies grew considerably in the three preceding cycles (Text-fig. 1), their cells divided frequently between injection and the time of killing. The corpora lutea of E₂ and E₁ showed the same autoradiographic characteristics as described above. They were displaced from the periphery according to their age. The central stroma with small islands of corpus luteum material consisted, therefore, of groups which showed heavily and lightly labelled cells, as well as small islands with unlabelled cells. These are thought to be rests of corpora lutea of E₀.

Cells of the large follicles in the periphery of the ovary were only lightly labelled. This is true for the follicle as well as the theca cells, both divided apparently frequently after the label was injected. A few small follicles could be found which had in their ring of follicle cells a single heavily labelled cell. This suggests that these cells have not divided, that the ring of cells in which they reside has not grown and the number of their cells has not increased in the 14 days following injection.

Many of the cells in the interfollicular, peripheral stroma were labelled, some heavily, some lightly (Pl. 2, Fig. 9). These cells were probably flash labelled in follicles which became contracted at varying times after the injection of the marker. Those that gave a heavy autoradiographic picture in E₃ did not divide any further, they remained undivided in the contracted follicle. Those that were lightly labelled, either divided once or twice before the follicle became contracted, or they began to divide again at a later period when the cells had become part of the peripheral stroma (Pl. 2, Fig. 10).

**Ovaries injected in oestrus (E₀) and killed four cycles later (E₄). (E₀⋅⋅⋅⋅E₁⋅⋅⋅⋅E₂⋅⋅⋅⋅E₃⋅⋅⋅⋅E₄)**

The interval between injection and death varied between 17 and 22 days. Autoradiographs of three animals were examined in this group. The labelling
pattern of the follicles in this group is characteristic: cells of the large follicles show hardly any label, whereas heavily and lightly labelled single cells in small and growing follicles stand out in the periphery of the organ (Pl. 2, Fig. 11).

There are many heavily and lightly labelled cells in the peripheral stroma. The cells of the fresh corpora lutea are not labelled. Apparently the structures

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- **Heavily labelled cell**
- **Lighter label: cell divided once or a few times**
- **Diluted label: cell divided many times**

**Text-fig. 1.** Schematic illustration of: (1) the development of the different structures of the ovary in relation to time after flash labelling, and (2) label incorporation and label dilution.
that in E₄ become fresh corpora lutea were at the time of flash labelling still small follicles or growing ones (Text-fig. 1), their cells divided too many times in the interval for the label still to produce an autoradiographic image.

The older corpora lutea, lying in the centre of the organ are interesting to examine. Some small corpora lutea islands lying close to large vessels still have two types of labelled cells: heavily and lightly labelled ones. These probably are rests of corpora lutea of E₁ and E₂. However, most central stroma is only little labelled or not labelled at all.

It is remarkable that in some sections areas with very heavily labelled cells in the surface epithelium are still found.

Ovaries injected in oestrus (E₀) and killed five cycles later (E₅). (E₀····E₁····E₂····E₃····E₄····E₅)

The interval between injection and death varied between 21 and 28 days. Autoradiographs of three animals were examined in this group.

It was of interest to learn whether any of the cells which were flash labelled in E₀ could still be recognized 3 or 4 weeks later, after the ovary had completed five cycles. In all three specimens occasional very heavily labelled cells in the surface epithelium were noted. None of the large follicles had labelled cells. Some follicle cells of Type 3a and 3b follicles were labelled. These were ‘inactive’ labelled follicles, i.e. those which did not grow and did not degenerate, but awaited the stimulus to develop.

Many cells of the peripheral stroma were still labelled. This was in contrast to the central stroma (corpora lutea rests and islands), which contained almost no more labelled cells.

DISCUSSION

The autoradiographs of ovaries, prepared at different time intervals after flash labelling, show that it is possible to mark cells in distinct cell groups in the ovary and to follow their growth, movement and disappearance from cycle to cycle for a considerable period of time.

From their appearance the following developments are suggested: the mature follicles which ovulate, are apparently already large follicles (Type 7) in the previous oestrus. It seems that smaller follicles do not usually, within a single cycle, become the ovulating follicle. This can be judged by the fact that the theca cells of Type 7 follicles, which label in oestrus at flash labelling, are just as heavily labelled in E₁ when this follicle has become a young corpus luteum. If the young corpus luteum of E₁ had originated from a follicle that in E₀ (at flash labelling time) was a growing (Type 3b or Type 4) or a young follicle (Type 5), these cells would have had to divide many times and their label would be diluted. This happens in fact with growing and young follicles that are flash labelled in E₀ and that become corpora lutea several cycles later: their theca cells indeed show a markedly diluted label, suggesting that these follicles grew in diameter, making it necessary for the ‘cover’, i.e. the theca
envelope, to enlarge too. Small follicles (Type 2 and Type 3a) and early growing follicles (Type 3b) remain unaltered in size for an unknown period of time. A certain number of those, that incorporate the label into one or two cells of the follicular ring in $E_0$, are found, even five cycles after flash labelling, still as small or early growing follicles with one or two cells in their follicle ring labelled. How long these follicles can remain in their 'inactive' condition before they receive the stimulus to grow was not determined.

However, it seems, that after a follicle has reached a certain stage of development it will not fall into an 'inactive' state again but either continue to grow uninterruptedly or degenerate. The critical stage after which the development continues uninterruptedly seems to be the Type 5 follicle.

Continuous growth is suggested by the following consideration: at flash labelling all large-sized follicles (Type 5 and larger) incorporate the label into many of their follicle cells. In the autoradiographs prepared at the following cycles all large follicles show diluted label in their follicle cells. If the large follicles were to remain inactive for some time at a certain size before growing further, heavily labelled cells would still be found in the population of their follicle cells. However, heavily labelled follicle cells in Type 5 or larger follicles have rarely been found in any of the later cycles, i.e. their follicle cells divided continuously after flash labelling.

The rather frequent degeneration of the Type 5 follicle is suggested by the occurrence of many 'contracted' peripheral follicles in which the follicle cells do not divide any further (heavily labelled cells), the oocyte degenerates, the zona pellucida rest disappears, the central cavity becomes obliterated and finally interfollicular peripheral stroma results. The development of these events can be followed in the autoradiographs of the consecutive cycles.

The interfollicular stroma persists in the periphery for considerable periods of time. The cells which become part of it, after a follicle contracts, remain intact: they do not degenerate, their nuclei remain morphologically unaltered. After varying time intervals these cells, as part of the stroma, begin to divide again.

It was possible in the autoradiographs to follow the formation, the displacement and final disappearance of the corpora lutea. A recently ruptured follicle seems to receive, with the quickly forming and invading blood vessels, cells which are not, before rupture, part of the follicle. In addition, cells of the theca interna invade the body of the young corpus luteum and intermix with the former follicle cells. Within the next cycle the corpus luteum is displaced from the periphery. In the second cycle it is further displaced, lying close to the central blood vessels, where it begins to shrink considerably. In the third cycle, its size is further reduced and it is often recognizable only as a small island of the central stroma. After four cycles, it usually has disappeared without a trace.

During the examination of the autoradiographs one fact stood out and, though it is connected with the failure to label, it seems of significance. In none of the many autoradiographs of serially sectioned ovaries of adult mice has a single labelled oocyte been seen. This failure to incorporate tritiated thymidine supports strongly the opinion that oogenesis—formation of new oocytes—does not take place in the adult mouse during oestrus.
ACKNOWLEDGMENTS

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