FOLLICLE GROWTH IN THE IMMATURE MOUSE OVARY

By

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Abstract

The growth of follicles in the immature mouse ovary was investigated in autoradiographs prepared after pulse-labelling with tritiated thymidine. Three parameters, which determine follicle growth were estimated:

1) The number of follicles present in the ovary at different ages.
2) The time it takes follicles to grow from one stage of development to another. This was calculated from the total number of granulosa cells in these stages and from their doubling times. The doubling time of granulosa cells was determined from their labelling index and the duration of their DNA-synthesis phase.
3) The number of follicles, which start on their development at different ages.

It was found, that the follicle development is not constant in the period from birth to maturity, but varies considerably. More follicles start to grow in the young than in the older immature mouse. Moreover the follicles grow faster early in life than later. The development from a follicle with one layer of granulosa cells to one with several layers and antrum formation takes about 10 days in the first half of the immature period, while it takes about 16 days as the animal approaches maturity. It was furthermore shown, that about 850 follicles start to grow in the immature period.

The morphology of the ovary changes considerably between birth and maturity. At birth the ovary consists mainly of small oocytes and stroma cells, while at maturity, 5 weeks later, follicles in all stages of development are present in the ovary. Moreover large follicles are seen in various degrees of degeneration, and a considerable amount of stroma has formed, mainly from degenerated follicles (Peters 1969). These changes in the morphology of the ovary during the immature period are a result of the continuous development and degeneration of follicles.
To investigate follicle growth in the ovary of the immature mouse several methods have been applied. With histological methods the number of follicles in different stages of development present in the ovary at different ages can be determined (Brambell 1928). Engle (1931) made an attempt to measure growth rates of follicles, by recording the age at which certain developmental stages first were seen during the immature period. By this method only the growth rate of the fastest growing follicles can be determined and the method can only be used in the first half of the immature period, as later no new, i.e. more advanced, stages of follicle development are seen. The time it takes follicles to reach certain stages of development, i.e. the dynamics of follicle growth, can not be investigated by these methods.

With the introduction of labelled thymidine and the use of autoradiography, it has become possible to describe in detail the cell population kinetics of tissues. The kinetics of the cell populations in the one month old mouse ovary and methods to determine growth rates of follicles have recently been described (Pedersen 1969, in press).

It is the purpose of this paper to describe the growth of follicles in the immature mouse ovary using this method, and to compare growth rates of follicles at different ages within this period.

**PRINCIPLES AND DEFINITIONS**

**Classification of follicles**

The classification of follicles proposed by Pedersen & Peters (1968) has been used in the present investigation. The follicles are divided into different types according to the number of granulosa cells on the largest cross sections of the follicles.

To describe the growth rate of follicles it is more practical to use this classification as a system of compartments, through which the follicles move during their development and where every type of follicle can be considered as a single compartment. The entry into the compartment is defined by a number of granulosa cells, and the exit from the compartment by another and larger number of granulosa cells.

The total number of granulosa cells in a follicle \( N \) can be calculated from the number seen on the largest cross section \( n \) and the diameters of the follicle by the following formula (Pedersen 1969, in press):

\[
N = n \frac{4/3 (R^3 - r^3)}{(2 r_c + f - 2p) (R^2 - r^2)}
\]

where \( R \) = diameter of the whole follicle  
\( r \) = diameter of the oocyte  
\( r_c \) = diameter of nucleus of the granulosa cells  
\( f \) = section thickness  
\( p \) = the height of the smallest segment of a granulosa cell, which is visible.

In one hundred follicles of varying sizes, the number of cells on the largest cross section and the diameter of the follicle was determined and the total number of cells
in the follicles was calculated from these values. From these results, it was possible to estimate the relation between the number of granulosa cells on the largest cross section and in the whole follicle (Pedersen 1969, in press).

The classification of follicles according to types and compartments is shown (Table 1). The number of cells on the largest cross section, which defines the types and compartments, and the corresponding number of cells in whole follicles are recorded.

Instead of describing the growth rates of follicles as the time it takes to reach certain developmental stages, the transit times of the follicles have been used as an expression of growth rates. The transit time is defined as the time it takes a follicle to grow through a compartment.

Principles for the description of follicle growth

The description of the growth of follicles involves: 1) the number of follicles in the different compartments (differential counts) and 2) the transit times of the follicles in these compartments. The results of the differential counts and the transit times obtained at different ages are then compared.

Differential counts

The number of follicles in the different stages of development is counted in every fifth section of each ovary. The follicles were classified according to the number of cells on the largest cross section, while the morphology was not taken into consideration.

Table 1.

Classification of follicles into compartments and types according to the number of granulosa cells on the largest cross section (thickness 5 µm) and the corresponding calculated number of granulosa cells in whole follicle.

<table>
<thead>
<tr>
<th></th>
<th>Number of cells on largest cross section</th>
<th>Corresponding number of cells in the whole follicle</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Compartment of type 3b</strong></td>
<td>20</td>
<td>90</td>
</tr>
<tr>
<td>Medium follicles</td>
<td>40</td>
<td>220</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>410</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>635</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>905</td>
</tr>
<tr>
<td><strong>Compartment of type 5a</strong></td>
<td>150</td>
<td>1420</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>2100</td>
</tr>
<tr>
<td><strong>Compartment of type 5b</strong></td>
<td>300</td>
<td>3000</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>5950</td>
</tr>
<tr>
<td><strong>Compartment of type 6</strong></td>
<td>500</td>
<td>8050</td>
</tr>
<tr>
<td></td>
<td>600</td>
<td>10400</td>
</tr>
<tr>
<td><strong>Compartment of type 7</strong></td>
<td>800</td>
<td>15600</td>
</tr>
</tbody>
</table>
Transit times of follicles

The transit time of follicles in a compartment can be calculated from two parameters: 1) the total number of cells in follicles corresponding to the entry and the exit from the compartment (Table 1) and 2) the doubling time of the granulosa cells in follicles belonging to this compartment.

Doubling time of granulosa cells

The doubling times of granulosa cells is defined as the time, it takes the cells of a given follicle to double their number. The doubling time of granulosa cells in a compartment can be calculated from the following parameters, which can be determined in autoradiographs after injection of tritiated thymidine: a) the labelling index (l. i.) of the granulosa cells in this compartment, b) the duration of the DNA-synthesis phase (S-phase) of the individual granulosa cells.

Labelling index

The l. i. is defined as the number of labelled granulosa cells in percentage of total number of cells in a follicle. It is determined in the largest cross section of each follicle in autoradiographs prepared 1 hour after injection of tritiated thymidine.

Duration of S-phase

The duration of the S-phase can be determined in autoradiographs prepared after varying time intervals after pulse labelling by the method of labelled mitosis (Quastler & Sherman 1959; Takahashi 1966; Cleaver 1967).

MATERIAL AND METHODS

The growth of follicles has been investigated at the age of 7, 14, 21, 28 and 35 days.

Experiment 1

The differential counts and the l. i. of the follicles were determined: 5 Bagg mice at each of the age groups investigated were injected intraperitoneally with 5 μCi ³H-thymidine/g body weight. All mice were killed 1 h after the injection of the label, and autoradiographs were prepared.

Experiment 2

This was done in order to determine the duration of the S-phase in granulosa cells at different ages.

At the age of 28 days 50 mice were injected intraperitoneally with 50 μCi ³H-thymidine (³HTdR) each and killed after varying time intervals from 1 to 44 h. Autoradiographs were then prepared.

At the age of 21 days fifteen mice were injected with 40 μCi ³H-thymidine each and killed at intervals from 1 to 16 h after the injection.

* The ³H-thymidine was manufactured by Amersham Radiochemical Center, specific activity ranging from 3.6 Ci/mM to 5.0 Ci/mM.
At the age of 14 days fifteen mice were injected with 25 μCi $^{3}$H-thymidine each and killed at the same time intervals as the 21 day old mice.

**Autoradiographic procedure**

The ovaries were fixed in Bouin's solution, embedded in paraffine and serially sectioned at 5 μm. The sections were coated with photographic emulsion (Ilford K2 research emulsion). After varying exposure times ranging from 1 to 4 weeks the autoradiographs were developed and stained with haematoxylin-cosin.

**Counting procedure**

*Experiment 1.* Every fifth section of the ovaries was examined. The total number of granulosa cells and the number of labelled granulosa cells were counted in those cross sections of follicles which contained the nucleolus of the oocyte (These sections are for practical purposes identical with the largest cross section). Cells were considered labelled, if they had 4 or more grains over the nucleus (the background labelling was less than 0.5 grains per 100 μm²). The I.I. of each follicle was calculated.

At the same time differential counts of follicles were determined. By multiplying the results of the differential counts with 5 the number of follicles in the whole ovary was obtained.

*Experiment 2.* In each ovary labelled and unlabelled mitosis were counted in the different types of follicles. Only meta-, ana- and telophases were taken into consideration. Mitosis are rarely seen in the medium follicles. Therefore in type 3b, 4 and 5a follicles, mitosis belonging to several follicles had to be grouped together. When at least 25 mitosis in each of these types were examined, the percentage of labelled mitosis was calculated. In the large types of follicles at least 100 mitosis were examined. The results were plotted against the time after injection. On the curves of labelled mitosis thus obtained for the different types of follicles the duration of the S-phase of granulosa cells could then be measured (Quastler & Sherman 1959; Cleaver 1967; Pedersen 1969, in press).

**RESULTS**

**Differential counts of follicles**

The number of follicles of different types was determined in each ovary. Their mean number was then calculated for each age group (Table 2). The largest follicle found in the ovary of a seven day old mouse is a type 3b or an early type 4 with about 50–70 granulosa cells on its largest cross section. At the age of 14 days the follicle development has reached type 5a. The largest follicle at this age has about 140–160 cells on the largest cross section. At the age of 3 weeks the ovary in addition contains large follicles of type 5b and 6, while at later ages type 7 and eventually type 8 (preovulatory follicles) appear.

The number of the medium follicles shows only small variations from the time they first appear until the age of 28 days. Hereafter their number decreases. The number of large follicles is highest about day 21 and then falls (Table 2).

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Table 2.
Differential counts of follicles.
Number of follicles in one ovary at different ages.

<table>
<thead>
<tr>
<th>Age days</th>
<th>Small follicles</th>
<th></th>
<th>Medium follicles</th>
<th></th>
<th>Large follicles</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Type 3a Mean</td>
<td>S. E.</td>
<td>Type 3b Mean</td>
<td>S. E.</td>
<td>Type 4 Mean</td>
<td>S. E.</td>
</tr>
<tr>
<td>7</td>
<td>377</td>
<td>65</td>
<td>141</td>
<td>45</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>479</td>
<td>19</td>
<td>155</td>
<td>9</td>
<td>125</td>
<td>0</td>
</tr>
<tr>
<td>21</td>
<td>461</td>
<td>49</td>
<td>163</td>
<td>15</td>
<td>113</td>
<td>18</td>
</tr>
<tr>
<td>28</td>
<td>504</td>
<td>54</td>
<td>142</td>
<td>20</td>
<td>94</td>
<td>13</td>
</tr>
<tr>
<td>35</td>
<td>365</td>
<td>48</td>
<td>105</td>
<td>12</td>
<td>55</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
It can be concluded, that the number of developing follicles reaches a maximum in mice at the age of 21 days. The number decreases gradually and reaches a minimum at the age of 35 days.

**Labelling index of follicles**

The l. i. is needed for the calculation of the doubling time of the granulosa cells. The percentage of labelled cells is an expression of the number of cells which synthesize DNA at the time of injection.

Follicles which do not contain labelled cells at all, are regarded as non-proliferating, i.e. in a 'resting' stage, as their cells do not synthesize DNA.

The small follicles of type 2 very seldom contain labelled cells. The majority of these follicles therefore seem to be non-proliferating; they form the pool of follicles, from which follicles can start to develop. The small follicles of type 3a, however, often contain labelled cells in their largest cross sections, but the percentage of such 'labelled' cross sections varies with age (Table 3). The percentage is highest in 7 day old mice, decreases thereafter gradually and reaches a minimum in the 21 day old mice.

The small follicles and their growth have not been dealt with further in this investigation, as most of them are not growing actively.

Whatever the age of the mouse studied all medium and large follicles contain labelled cells in their largest cross section, i.e. they are all growing. The labelled cells are randomly distributed among the granulosa cells in follicles of type 3b to 6 incl. In type 7 follicles the labelled cells are located mainly around the cavity and in the cumulus, while the peripheral parts of the granulosa layer have none or very few labelled cells. Type 7 follicles are seldom seen in immature ovaries; they develop only shortly before maturity starts and their l. i. has not been determined.

There is a large difference between the l. i. of medium and large follicles within the same ovary (Fig. 1 a + b): medium follicles contain few labelled cells, their l. i. is low, while large follicles contain many labelled cells, i.e. they have a high l. i.

In ovaries of the same age, comparable follicles have the same labelling in-

**Table 3.**

The percentage of follicles of type 3a containing labelled cells on their largest cross section one hour after pulse labelling with $^3$HTdR.

<table>
<thead>
<tr>
<th>Age</th>
<th>7 days</th>
<th>14 days</th>
<th>21 days</th>
<th>28 days</th>
<th>35 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>% labelled follicles</td>
<td>73.8</td>
<td>49.4</td>
<td>34.1</td>
<td>35.0</td>
<td>38.1</td>
</tr>
<tr>
<td>$sd$ = 5.1</td>
<td>$sd$ = 2.3</td>
<td>$sd$ = 3.7</td>
<td>$sd$ = 4.1</td>
<td>$sd$ = 4.8</td>
<td></td>
</tr>
</tbody>
</table>

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Fig. 1 a + b.
Autoradiographs of ovaries from 21 day old mice killed one hour after pulse-labelling with $^3$H-thymidine. In the medium follicles of type 3b (A), type 4 (B) and type 5a (C) only few labelled granulosa cells are seen. The large follicles of type 5b (D) and type 6 (E) contain many labelled granulosa cells. ($\times$ 100).
dices. When mice of different ages are compared, follicles in the same stage of development do not necessarily have the same l. i.

The relation between l. i. and the number of granulosa cells on the largest cross section at three different ages 7, 14 and 21 days is shown in Fig. 2. The type 3b follicles present in ovaries of 7 day old mice have a higher l. i. than follicles of the same type of the other ages. At day 14 also type 4 and 5a are found in the ovaries. Their l. i. is higher than in comparable follicles at the age of 21 days, but the large follicles seen at the age of 21 days, have the highest l. i. of all follicles seen up to this age.

The mean labelling indices for the different types of follicles at the ages investigated have been calculated (Table 4). Type 3b follicles, i.e. those which have just begun to develop, have their highest l. i. (20.8 %) in 7 day old mice. Later in the infant period the l. i. of this type is considerably lower reaching

![Fig. 2.](image)

Labelling index (l. i.) of different types of follicles one hour after injection of 
$^3$H-thymidine in 7, 14 and 21 day old mice.

<table>
<thead>
<tr>
<th>Table 4.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean labelling index of the different types of follicles in the ovary at different ages.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age days</th>
<th>Medium follicles</th>
<th>Large follicles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>type 3b %</td>
<td>type 4 %</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>7</td>
<td>20.8</td>
<td>—</td>
</tr>
<tr>
<td>14</td>
<td>13.2</td>
<td>10.8</td>
</tr>
<tr>
<td>21</td>
<td>8.5</td>
<td>8.4</td>
</tr>
<tr>
<td>28</td>
<td>7.4</td>
<td>7.4</td>
</tr>
<tr>
<td>35</td>
<td>8.3</td>
<td>8.3</td>
</tr>
</tbody>
</table>
a minimum (8.5 °/o) in 21 day old mice. Type 4 follicles were first seen in 14 day old mice; at this age their l. i. (10.3 °/o) is somewhat higher than in older mice. The l. i. of type 5a is almost unchanged in this period. The large follicles have a high l. i. (30 °/o) from the time they first appear on day 21. The l. i. even increases a little until maturity is reached (35–38 °/o).

**Duration of the S-phase**

To determine the duration of the S-phase in the granulosa cells of different follicles the percentage of labelled mitosis has been determined in autoradiographs prepared at different time intervals after injection of ³H-thymidine (³HTdR). The percentage of labelled mitosis is plotted against the time after injection (Fig. 3). On the curve the duration of the S-phase can be measured as the time interval on the abscissa corresponding to 50 °/o labelling on the ascending and descending part of the first wave. The S-phase of granulosa cells belonging to follicles in different stages of development varies considerably. In 28 day old mice the S-phase of granulosa cells in type 3b follicles has a mean value of 9.8 h. In type 4 it is 11.2 h and in type 5a 10.3 h. The S-phase of the granulosa cells in large follicles is shorter, the mean value averages 6.8 h in both type 5b and 6.

The curve of labelled mitosis was not worked out in detail for the other age groups, but it was tested, whether there are differences between the shape and position of the curves in 28 day old mice and other ages. This was done by making a number of observations of the percentage of labelled mitosis at time

![Image](https://example.com/image_url)

*Fig. 3.*

The percentage of labelled mitosis among granulosa cells in type 6 and type 4 follicles at different ages as a function of time after a pulse label with ³HTdR.
intervals corresponding to the ascending and descending part of the first wave. Fig. 3 shows two examples of such tests. In the first example, observations in type 6 follicles at the age of 21 days are shown as solid squares. These observations do not differ significantly from the values obtained in 28 day old mice (open circles). This infers, that the wave of labelled mitosis, and therefore also the duration of the S-phase in granulosa cells in type 6 follicles is identical in these ages. The second example shows, that the S-phase is the same in type 4 follicles at the age of 14 and 28 days. Also the curves of labelled mitosis in the other types of follicles, 3b, 5a and 5b were identical in the ages of 14 and 21 and 28 days.

It can be concluded, that the duration of the S-phase of granulosa cells in the same type of follicle does not vary at different ages. This is in contrast to the l. i. of follicles of type 3b and 4 which was found to vary considerably.

Doubling time of granulosa cells

The doubling time of granulosa cells can be calculated from the following formula, under the assumption, that the cell population is growing exponentially (Cleaver 1965). The latter condition is fulfilled in the follicles (Pedersen 1969, in press).

\[ L. I. = (\exp \frac{t_s \ln 2}{T_D} - 1) (\exp \frac{t_2 \ln 2}{T_D}) \]

\( t_s \) = duration of S-phase
\( T_D \) = doubling time
\( t_2 \) = the duration of G2-phase + half of the mitosis phase.

L. i. and \( t_s \) has already been determined. The value of \( t_2 \) can also be determined on the curve of labelled mitosis: \( t_2 \) is represented by the time interval between injection and the time when 50 \% labelled mitosis are first seen. In the medium follicles this is 2.0 h, and in the large follicles 1.8 h. These values were found not to vary with the age of the mouse.

The doubling times of each type of follicle in the age groups investigated are recorded in Table 5. At all ages there is a marked difference between medium and large follicles: the doubling time is much shorter in the large than in medium follicles. The values for the large follicles do not vary with age. However, the doubling times of some of the medium follicles show considerable variations, not only from one type to another, but also within the same type at different ages. Granulosa cells in a type 3b follicle, f.i. double their number in 38 h in 7 day old animals, while it takes more than twice as long (84 h) in 21 day old mice. The doubling time in other types varies less.

Transit times of follicles

The time it takes a follicle to grow through a compartment, the transit time
Table 5.
Doubling time of granulosa cells in different types of follicles at different ages in the immature period.

<table>
<thead>
<tr>
<th>Age days</th>
<th>Medium follicles</th>
<th>Large follicles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>type 3b hours</td>
<td>type 4 hours</td>
</tr>
<tr>
<td></td>
<td>type 5b hours</td>
<td>type 6 hours</td>
</tr>
<tr>
<td>7</td>
<td>38</td>
<td>—</td>
</tr>
<tr>
<td>14</td>
<td>54</td>
<td>80</td>
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<tr>
<td>21</td>
<td>84</td>
<td>99</td>
</tr>
<tr>
<td>28</td>
<td>76</td>
<td>110</td>
</tr>
<tr>
<td>35</td>
<td>66</td>
<td>101</td>
</tr>
</tbody>
</table>

\[ T_F = \frac{T_D (\ln N_0 - \ln N_i)}{\ln 2} \]

where \( N_i \) = total number of cells in follicles entering a compartment
\( N_0 \) = total number of cells in follicles leaving a compartment.

The transit times of follicles at the different ages are recorded in Table 6.

The transit times show a similar trend in their variation as the doubling times: they are almost unchanged between the ages of 21 and 35 days for all types of follicles, while they are considerably shorter in 7 and 14 day old mice.

Between the ages of 21 and 35 days it takes about 7 days for a follicle to develop from one with 20 cells to one with 60 cells on the largest cross section, i.e. it takes 7 days to grow through the compartment of type 3b. At day 7 a follicle takes only \( \frac{3}{2} \) days to grow through the same compartment.

Table 6.
Transit time of follicles at different ages in the immature period.

<table>
<thead>
<tr>
<th>Age days</th>
<th>Medium follicles</th>
<th>Large follicles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>type 3b hours</td>
<td>type 4 hours</td>
</tr>
<tr>
<td></td>
<td>type 5b hours</td>
<td>type 6 hours</td>
</tr>
<tr>
<td>7</td>
<td>83</td>
<td>—</td>
</tr>
<tr>
<td>14</td>
<td>119</td>
<td>91</td>
</tr>
<tr>
<td>21</td>
<td>185</td>
<td>113</td>
</tr>
<tr>
<td>28</td>
<td>167</td>
<td>126</td>
</tr>
<tr>
<td>35</td>
<td>145</td>
<td>115</td>
</tr>
</tbody>
</table>

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The transit times of large follicles are much shorter than that of the medium follicles. It takes, f. i. only 14 h for a follicle to grow through the compartment of type 6. The transit times of large follicles show no variation with age.

The age at which a certain type of follicle first is seen in the ovaries correlates well with the calculated transit times, and acts as a test on the reliability of these transit times. The largest follicle on day 7 is a follicle with 50–70 cells on its largest cross section (a type 3b). Using the calculated transit times, it can be predicted, that on day 14 the largest follicle will have about 150–200 cells on its largest cross section (a type 5a), which is in good agreement with the actual findings: the largest follicle found at this age had some 160 cells on the largest cross section.

Follicle kinetics

It is possible to calculate the time it takes a follicle to grow from one stage of development to another by adding the transit times of the compartments the follicle passes through: The development from an early type 3b follicle to a late type 6 takes about 16 days in 3 to 5 week old mice. In a younger animal this development goes faster, f. i. a follicle starting to grow as a type 3b at the age of 7 days takes only 10–12 days to develop to a type 6. This shortening of the development is due to shorter transit times of the compartments of type 3b and 4 follicles.

The growth of follicles in the compartments of type 3b and 4 is assumed to be in a steady state, i. e. the number of follicles which enter (the inflow) equals the number leaving the compartments (the outflow), and no follicles are lost from the compartment. The assumption of steady state is supported by the observations, that the total number of follicles in these compartments does not change considerably within the first 4 weeks, and that degeneration of these types are very rarely seen.

The inflow into a compartment, defined as the number of follicles which enter the compartment per hour, can be calculated by dividing the number of follicles present in the compartment by the transit time of this compartment. This has been determined for the type 3b and 4 compartments at different ages (Table 7). The inflow into type 3b is not constant. As it represents the beginning of active follicle development, it is realized, that the number of follicles which start to grow at different ages during the immature period varies considerably. In a seven day old mouse the number of follicles that begin their development is largest; it is more than twice as large (1.7 follicle per hour) as in a 35 day old mouse (0.8 follicles per hour).

Considering the whole immature period it is noted, that many follicles begin to grow in the early part of the period and that the number which starts development decreases as the mouse approaches maturity.

The inflow into the compartment of type 4 is of the same order of magnitude
as the inflow into type 3b at comparable ages. This fact underlines the assumption, that steady state conditions do exist in the compartment of type 3b follicles.

In the compartments of large follicles, however, steady state conditions do not exist, as there is a loss of follicles due to degeneration (which is easily seen in histological preparations). The flow through these compartments therefore cannot be estimated.

If it is assumed, that the percentage of follicles degenerating in the compartments of type 5b and 6 is constant, then the total number of type 5b and 6 follicles found in 21, 28 and 35 day old mice would reflect the number of follicles, which started to grow in 7, 14 and 21 day old mice. This is actually found: the highest number of follicles (during the immature period) that start to develop is found in the 7 day old mouse. As it takes ca. 12 days for these follicles to grow to a type 5b and 6 follicle, a maximum in the number of large follicles should be expected in the ovaries of 21 day old animals. This is actually the case (Table 3): at no other time before maturity is such a large number of large follicles present in the ovary \((38 + 13 = 51)\) as on day 21.

The number of follicles that start to grow decreases progressively in 2 and 3 weeks old mice and 2 weeks later the number of large follicles is also smaller (41 and 25 respectively). Furthermore the relation between follicles starting to grow in 7, 14 and 21 day old animals \((1.7:1.3:0.9)\) corresponds completely to the number of large follicles found in ovaries of 21, 28 and 35 days old animals \((51:41:25\) or \(1.7:3.0:0.8)\).

This comparison of the number of follicles starting and the number of large follicles found 2 weeks later, acts as a control on the calculations performed, especially the calculations of the transit times. The good agreement between the results confirm the calculations.

\[\text{Table 7.}\]
The number of follicles entering the compartment of type 3b (inflow into type 3b) and the compartment of type 4 (inflow type 4) per hour at different ages.

<table>
<thead>
<tr>
<th></th>
<th>7 days</th>
<th>14 days</th>
<th>21 days</th>
<th>28 days</th>
<th>35 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflow into type 3b</td>
<td>1.7</td>
<td>1.3</td>
<td>0.9</td>
<td>0.9</td>
<td>0.8</td>
</tr>
<tr>
<td>Inflow into type 4</td>
<td>—</td>
<td>1.4</td>
<td>1.0</td>
<td>0.8</td>
<td>0.6</td>
</tr>
</tbody>
</table>
DISCUSSION

In the infant mouse a continuous growth and development of follicles take place in the ovary. However, the number of follicles that begin to grow and their speed of growth is not constant, but varies with age. It is early in life that the greatest growth activity takes place: already on day 7 more follicles start to grow faster than at any other time in the immature period. This leads to an unusually high number of large follicles to be present in the ovaries of 21 day old mice. However, the hormonal interplay in the immature mouse does not support further follicle development yet, and they degenerate. Thus the apparent paradox of seeing an unusually high number of large 'healthy' follicles and at the same time an unusually high number of degenerating follicles in the 3 week old mouse can be explained by the fact that 2 weeks earlier many follicles started to grow and grew quickly, but that their development beyond a certain stage cannot as yet be supported.

After the first week of age the number of 'starting' follicles and the speed of their development decreases gradually, reaches a low value at 21 days and thereafter remains constant during the late immature period. These variations in the growth pattern result in the variations in the number of large follicles found in the late immature mouse.

As the number of follicles starting to grow every hour at certain ages has been determined, it is possible to estimate the total number of follicles, which start to grow during the whole immature period. In the first week of life about 140 follicles start to develop, namely the number of medium follicles seen on day 7 (Table 2). In the first half of the week between day 7 and day 14 it is assumed, that an average of 1.7 follicles start to grow every hour (Table 7), while in the last half of this week an average of only 1.3 follicles begin their development every hour. Thus in the week from day 7 to day 14 about 250 follicles start to grow, and within the first two weeks of life about 390 follicles (140 + 250). In the same way, it can be calculated, that from birth to day 21 about 600 follicles start, and that in the whole immature period (from birth to day 35) about 850 follicles start to grow.

As the degeneration of medium follicles is negligible, and as the development from an early medium follicle to a large one does take more than 14 days, it can be concluded that the 600 follicles starting to grow within the first 3 weeks become large follicles before maturity starts. The vast majority of these degenerates within the late immature period (from day 21 to 35). This means, that in average between one and two large follicles start to degenerate every hour in this period.

It can be concluded, that the ovary of the immature mouse is far from a dormant organ, but one in which a rapid and continuous growth and development of follicles take place.
REFERENCES


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