Chapter - II

A Study of Mitotic Index, Cell Size and Weight in Differentiating Liver of Chick
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INTRODUCTION

The first visible liver primordium is seen, in the chick, at the end of the second day of incubation with 16 to 23 somites. Anastomoses between the trabeculae of the cranial and caudal diverticula may occur before the end of the 3rd day or early in the fourth day. The proliferation of liver trabeculae is accompanied by the sprouting of capillaries from the ductus venosus (Romanoff, 1960). During the 4th day of development, the lateral anastomoses become so numerous that a network of liver tissue forms an open meshed cylinder around the ductus venosus. On the 5th day the liver is smooth externally and has the appearance of a compact organ, although its interior is still trabecular.

The primordial liver consists of undifferentiated cells, which pass through some basic functional activities such as cellular proliferation, differentiation, migration along with the secretion and accumulation of synthetic materials. The liver's growth rate is highest, of course, during the early stages of its development as indicated by relative weight increments. Several authors such as Schmalhausen (1929), Tamiya (1927b) and Dumm and Levy (1949) studied the growth
rate by measuring the wet weight and dry weight of the liver and found that growth rate gradually increases from 4th to 8th day of incubation. After 11th or 12th day, when the liver weighs from 75 to 100 mg, the growth rate decreases rapidly. But no attempt has yet been made to determine the growth rate of liver on the basis of cell proliferation. Growth of an organ, particularly in the embryonic stages, mainly depends on the rate of cell proliferation, increase of size of individual cells and synthesis and accumulation of cellular constituents resulting in the increase of weight.

The present investigation concerns with the survey of the mitotic index, cell size, wet weight and dry weight at different stages of the ontogeny of liver. The study will throw some light on the rate of cell proliferation and growth of liver at different stages of development. Moreover, a gross picture can be obtained regarding the rate of the formation of blood cells in the embryonic liver.

**MATERIALS AND METHODS**

The study is based on white leghorn embryos incubated at a constant temperature of 38°C with 75% humidity on an average. The liver of the embryos of different developmental stages were dissected out and fixed in alcoholic Bouin's fluid. After routine process of embedding in paraffin, serial sections were made at 10 μ and stained in Heidenhain's haematoxylin. Mitotic index was ascertained by considering
the dividing cells and nondividing ones in the tissue (Bertlanffy and Lau, 1963). Counting of cells was followed by the method of McKeehan (1951) by taking different areas in the sections.

The determination of the cell size was made from double stained section with haematoxylin and eosin. In this study generally the nondividing parenchymal cells of liver were taken into account. At least hundred cells of each embryo had been studied. Average wet weight and dry weight of each liver had also been estimated. The rate of growth was calculated by using the formula $V = \frac{dW}{dt} \cdot \frac{1}{W}$, where $V$ is the specific rate of growth and $W$ is the average weight of liver at any given time, $t$.

Along with these a preliminary survey of the rate of formation of blood cells in the liver, at different stages of development, has been made. In all of these studies at least five embryos of each stage had been considered.

**OBSERVATIONS**

A. **Mitotic Index and Hematopoiesis**:

The different aspects of the study viz. cell number, dividing cells, nondividing cells, and mitotic index in the liver at different ages of the embryos have been shown in Table - 1.
Table - 1.

Mitotic index in the developing liver of chick

<table>
<thead>
<tr>
<th></th>
<th>4 days</th>
<th>6 days</th>
<th>8 days</th>
<th>10 days</th>
<th>12 days</th>
<th>14 days</th>
<th>16 days</th>
<th>18 days</th>
<th>20 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of dividing cells</td>
<td>17</td>
<td>47</td>
<td>148</td>
<td>189</td>
<td>35</td>
<td>69</td>
<td>18</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>No. of nondividing cells</td>
<td>1302</td>
<td>3001</td>
<td>3984</td>
<td>5679</td>
<td>2064</td>
<td>3647</td>
<td>1776</td>
<td>1696</td>
<td>1410</td>
</tr>
<tr>
<td>Total cells</td>
<td>1319</td>
<td>3048</td>
<td>4132</td>
<td>5868</td>
<td>2099</td>
<td>3716</td>
<td>1794</td>
<td>1704</td>
<td>1416</td>
</tr>
<tr>
<td>Mitotic Index</td>
<td>1.28</td>
<td>1.54</td>
<td>3.60</td>
<td>3.22</td>
<td>1.67</td>
<td>1.85</td>
<td>1.0</td>
<td>0.47</td>
<td>0.42</td>
</tr>
</tbody>
</table>

Observation from 4th to 8th Day's Embryos:

From the table - 1 it may be observed that the mitotic rate in the developing liver is gradually increased during this period and it is highest in the 8th day of incubation, when it reached 3.60. In the 4th and 6th day, the mitotic figures are 1.28 and 1.54 respectively.

Formation of blood cells starts at the end of this period i.e. at about 7th or 8th day of incubation.

Observation from 8th to 14th Day's Embryos:

During this period i.e. after 8th day, the mitotic index remains more or less equal up to 10th day and then rapidly decreases up to 12th day of incubation, when the figure is 1.67. After this there is a slight increase at the 14th day, and the mitotic index is 1.85.
Formation of blood cells is not so prominent in the initial stages of this period, but at about 14th day huge number of developing blood cells are found here and there, particularly in the perivascular connective tissue.

Observation from 14th to 20th Day’s Embryos:

The rate of mitosis decreases gradually during this period and the figures are 1.00, 0.47 and 0.42 in the 16th, 18th and 20th days respectively.

Hematopoiesis gradually decreases during this period and blood cell formation is very low at the 20th day of incubation.

B. Cell Size:

The Table - II shows that the average cell size of the liver parenchyma increased in the early stages of development i.e. from 4 days to 8 days of incubation, and then there is a period of stagnancy ranging from 8 to 14 days. From 14 days a second increases of liver cell size occurs which continued up to 20 days of incubation.

Table - II

Average cell size of liver parenchyma(in square micromillimeter) at different stages of development

<table>
<thead>
<tr>
<th>Days of incubation</th>
<th>4 days</th>
<th>6 days</th>
<th>8 days</th>
<th>10 days</th>
<th>12 days</th>
<th>14 days</th>
<th>16 days</th>
<th>18 days</th>
<th>20 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average cell size in sq μm with S.E.</td>
<td>57±1</td>
<td>68±2</td>
<td>93±2</td>
<td>91±2</td>
<td>95±2</td>
<td>91±2</td>
<td>111±1</td>
<td>129±3</td>
<td>149±3</td>
</tr>
</tbody>
</table>
C. Wet Weight and Dry Weight:

Although the wet weight and dry weight of the liver are increased throughout the whole incubation period, but their trends vary at different stages of development. It may be seen that the increase of both wet weight and dry weight gradually becomes higher up to 12 days, when the value reached its peak, and after 12 days the value falls at 14 days of incubation. Thus there is a fall in wet weight from .097 at 12 days to .003 at 14 days, and that of dry weight from .125 at 12 days to .002 at 14 days of incubation (Table - III). After 14 days the values of the rate become higher at 16 days and then become lower at 18 days and slightly increased at 20 days of incubation.

Table - III

Average wet weight, dry weight and rate of growth of the developing liver of chick embryo

<table>
<thead>
<tr>
<th>Days of incubation</th>
<th>6 days</th>
<th>8 days</th>
<th>10 days</th>
<th>12 days</th>
<th>14 days</th>
<th>16 days</th>
<th>18 days</th>
<th>20 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average wet weight in mg.</td>
<td>2.81</td>
<td>3.85</td>
<td>13.5</td>
<td>76.62</td>
<td>87.73</td>
<td>253.6</td>
<td>258.1</td>
<td>282.83</td>
</tr>
<tr>
<td>Rate of increase of wet weight</td>
<td>.0077</td>
<td>.052</td>
<td>.097</td>
<td>.003</td>
<td>.039</td>
<td>.003</td>
<td>.001</td>
<td></td>
</tr>
<tr>
<td>Average dry weight in mg</td>
<td>.36</td>
<td>.5</td>
<td>2.6</td>
<td>18.22</td>
<td>20.36</td>
<td>63.05</td>
<td>64.1</td>
<td>123.77</td>
</tr>
<tr>
<td>Rate of increase of dry weight</td>
<td>.0081</td>
<td>.087</td>
<td>.125</td>
<td>.002</td>
<td>.043</td>
<td>.003</td>
<td>.019</td>
<td></td>
</tr>
</tbody>
</table>
DISCUSSION

In analysing the above results the growth of liver can broadly be divided into three phases. In the first phase ranging up to 8 days of incubation, there is gradual increase of mitotic index, increase of liver cell size and increased rate of weight. In the second phase of development i.e. from 8 days to 14 days of incubation, there is decline of mitotic index up to 12 days and then the index is slightly increased at 14 days. The rate of increase of weight gradually becomes higher up to 12 days and then falls at 14 days of incubation. The cell size remains more or less constant in this period. From 14 to 20 days of incubation, which comprises the third phase, mitotic index is gradually decreased, but the cell size is increased. The trend of increase of weight alternately become higher and lower in this period.

These three phases of development more or less correspond in time with the growth and differentiation of liver. Thus in the first phase the growth and differentiation of the liver is high as indicated by the beginning of secretion of bile between 7th to 9th, storing of glycogen at the 7th, appearance of lipid granules between 6th to 8th and the starting of the formation of blood cells at the 7th days of incubation, (Romanoff, 1960). Peptidase activity is increased from 5th to 8th day (Dumm and Levy, 1949). Mitotic index attains its highest level at the end of this period i.e. on the 8th day, when the figure is 3.60
Fig. 1. Mitotic Index in differentiating liver of chick.
(Table-I). Liver cell size and the increase of wet weight and dry weight also become higher in this period (Table-II and III).

During the second phase, the cell proliferation suddenly falls from 10th to 12th day, when the mitotic index falls from 3.22 to 1.67 (Table-I). The rate of increase of wet weight and dry weight also becomes lower after 12 days (Table-III). Tamiya (1927), Schmalhausen (1929) and Dumm and Levy (1949) also observed the sudden fall of growth rate of liver after 11th day, on the basis of wet weight and dry weight only. The present finding reveals that the above view is also true, when the study is based on mitotic index. The amount of glycogen decreases from the 9th to 12th day of incubation (Dalton, 1937b), but the lipid granules increase in size and number and the peptidase activity is increased. Cell size of liver parenchyma remains more or less constant from 8th to 14th day (Table-II). Hematopoiesis decreases from the 9th to 12th day of incubation, but after 12th day hematopoiesis is intense in the liver, and it is maximum at the 14th day. The slight increase of mitotic index at the 14th day might be due to this increase of hematopoiesis.

In the third phase, the mitotic index is gradually decreased up to the hatching time (Fig.1). During this period hematopoiesis is decreased. Accumulation of lipids along with the cholesterol, cholesterol-esters and fatty acids increased (Hanes, 1912; Dalton, 1934, 1937). Glycogen becomes evenly distributed throughout the liver after 14th day (Dalton, 1937b) as its
concentration rapidly rises (Potvin and Aron, 1927). A second increase of liver cell size occurs at about 16th day and is continued up to 20 days of incubation (Table-II). The rate of increase of weight rhythmically becomes higher and lower in this period.

From the above observations and discussions it becomes increasingly clear that the growth of liver in the first phase, which ranges from 4th to 8th days of incubation, depends on all of the three factors considered in the study, viz. proliferation of cells, increase of cell size and increase of weight. In the second phase of liver development, which ranges from 8th to 14th day, the growth mainly depends on mitotic index and increase of weight, as cell size remains more or less constant in this period; while in the third phase, which starts after 14th day and continued up to the hatching time, growth of liver is mainly due to the increase of cell size and increase of weight, as mitotic index falls below active growth level in this period. According to Price (1958), a tissue should be regarded as actively growing when its mitotic index exceeds 1.5. It has been observed that the mitotic index is below 1.5 after 14th days of incubation (Table-I). Thus considering the mitotic index, it may be said that the embryonic chick liver is not actively growing after 14th day of incubation. Moreover, the presence of mitotic peak at the 8th day, and the peak of the rate of weight increase at 12th day indicates that the cell proliferation plays a major role in the first phase of growth, and
weight increase plays a major role in the second phase of growth, while the increase of cell size contributes both in the first and third phases of growth of liver in chick.

Table IV

Analysis of variance

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>S.S.</th>
<th>M.S.</th>
<th>V.R.</th>
<th>Table value of F at 5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear</td>
<td>1</td>
<td>2.9124</td>
<td>2.9124</td>
<td>4.981</td>
<td>5.99</td>
</tr>
<tr>
<td>Quadratic</td>
<td>1</td>
<td>3.3596</td>
<td>3.3596</td>
<td>5.746</td>
<td>5.14</td>
</tr>
<tr>
<td>Error</td>
<td>6</td>
<td>3.5082</td>
<td>0.5847</td>
<td>5.363</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>9.7802</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

On the analysis of the data for mitotic indices at different ontogenic stages of liver, it has been found that a nine point orthogonal polygon of second degree may be fit (Table-IV). From the analysis of variance table (Table-IV) it can be observed that a second degree curve is just significant at 5% level. A comparative graph from the expected and observed values of Y (mitotic index) has been drawn (Fig.1).

It has been claimed that there are two distinct embryonic periods during which blood is formed in the liver of chick. The first begins on the 7th and the second on the 11th day of incubation. This process reaches its maximum on the 14th or 15th day and gradually ceases before the end of incubation (Haff, 1914).
This claim was supported by Sandreuter (1951). The present investigation is fully in agreement with this view, as two periods of hematopoiesis in the liver have been recognised, one beginning at 8th day and the other at about 12th day, which attains its peak on the 14th and then gradually ceases on the 20th day of incubation.

**SUMMARY**

1. Mitotic index, cell size, wet weight, dry weight and the formation of blood, at different developmental stages of liver in chick, have been studied.

2. It is found that mitotic index is gradually increased up to 8th day, when it reaches its peak and then decreased up to 12th day and again rises at 14th day. Thereafter it is gradually decreased up to the end of the incubation period. Cell size increased from 4 to 8 days and then there is period of stagnancy from 8 to 14 days; after which the cell size increased up to 20 days of incubation. The rate of increase of both wet weight and dry weight increased up to 12 days and then falls at 14 days of incubation. After 14 days the rate of weight increase rhythmically becomes higher and lower up to 20 days of incubation.

3. The growth of chick liver can broadly be divided into three phases - the first phase from 4 to 8 days, the second phase from 8 to 14 days and the third phase from 14 to 20 days of incubation. It has been observed that the mitotic index plays a major role in the first phase; rate of increase of weight
plays a major role in the second phase; while the increase of cell size contributes, both in the first and in the third phases of growth. These three phases more or less correspond in time with the growth and differentiation of liver in chick.

4. Formation of blood in the developing liver is characterized by two periods; one starts at about 7th and the other at about 11th or 12th day of incubation and then it is gradually decreased up to the 20th day.

5. The regression of mitotic activity of liver cells has a quadratic trend.

6. The significance of these findings has been discussed.

REFERENCES


