Growth and Function in the Foetal Liver

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INTRODUCTION

Many investigators have demonstrated the decline which takes place in the relative weight of the liver during the development of the mammalian foetus; Carlyle (1945) and Wallace (1945) in the sheep, Jackson (1909) in man, Lowrey (1911) in the pig, Williamson (1948) and Addis & Gray (1950) in the rat, Latimer & Corder (1948) in the dog, and Latimer (1948) in the cat. The present work is an attempt to find the cause underlying this decline. Since the foetal liver contains not only hepatic tissue but also much haemopoietic tissue, it was first necessary to determine whether the decrease in the relative liver-weight represents a real diminution of true hepatic tissue or whether it simply reflects the progressive shift of haemopoiesis from liver to skeleton. In order to test these alternatives a method was devised for determining the total amount of true hepatic tissue, i.e. the total number of hepatic cells, in a given foetal liver, and applied to a series of foetuses covering the developmental period.

MATERIAL AND METHODS

The material investigated was a series of fifty-one sheep foetuses ranging in length from 28 to 530 mm. These were collected from the dams immediately after slaughter. Dams were of many different breeds. The following measurements were made.

1. Forehead-rump length. This was measured to the nearest millimetre using the technique of Winters & Feuffel (1936).
2. Body-weight. Surface water was removed and the foetus weighed with the umbilical cord cut short.
3. Weight of liver. The liver was dissected clear of fascia and the gall bladder removed. Weighings were made after removal of surface water with blotting paper.
4. Volume of liver. Before this measurement the liver was fixed in 10 per cent. formalin. In some cases the liver was placed in a syphon apparatus and the volume of the displaced water measured. In others the volume was determined by successive weighings in air and water.

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The forehead-rump length was used to calculate the age of each embryo by means of the chart constructed from collected data by Barcroft (1946). This chart combines data obtained from many different breeds. From the scatter of the data given by Barcroft it may be inferred that the probable errors are as follows:

<table>
<thead>
<tr>
<th>Forehead-rump length (mm)</th>
<th>Estimated age (days)</th>
<th>Standard error (days)</th>
<th>Coeff. of var. (%)</th>
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<tbody>
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<td>3.6</td>
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<tr>
<td>450</td>
<td>138</td>
<td>8.3</td>
<td>6.0</td>
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</table>

Preparation of liver

A rectangular block was cut from the fixed liver, one of its faces cut with parallel edges, and the length of this face measured with an accuracy of 0.1 mm. by means of a microscope with a movable stage fitted with verniers. The block was dehydrated, embedded in paraffin, and sections cut at 10 μ. The measured edge on the remainder of the block was remeasured by the same method and the linear shrinkage factor,

\[
\frac{\text{length after embedding}}{\text{length after fixation}}
\]

was calculated. The volume shrinkage factor,

\[
\frac{\text{volume after embedding}}{\text{volume after fixation}}
\]

was calculated as the cube of the linear shrinkage factor. Six blocks were prepared in a batch and the average of the individual shrinkage factors was applied to the whole batch. The standard deviation of the average was used to estimate the error.

Measurement of section thickness

Sections were stained with haematoxylin and eosin and mounted in DPX. The thickness was then determined by focusing on the upper and lower surfaces of the section using a Leitz Ortholux microscope with an oil-immersion apochromatic objective × 90, N.A. 1.30, and × 12 periplanatic eyepiece. Readings were taken to the nearest 0.5 μ. The depth of focus of the optical combination is given by the maker as 0.8 μ and this figure was subtracted from all the thickness measurements made. Three independent determinations of thickness were made on a small marked part of each section. The lowest and highest of these in general differed by not more than 2 μ. The standard error of the average of the three measurements was within 0.5 μ in all cases. The method of Marengo (1944) was found to be unsuitable for this investigation owing to the considerable variation in the thickness of sections cut at the same time on the same microtome and to
the fact that the thickness measurements would have to be made on sections other than those used for subsequent counting.

**Hepatic cell count**

The number of hepatic cell nuclei in each of two areas of a section was counted using a × 90 oil-immersion objective. Both of the chosen areas lay within the marked part of the section whose thickness had been determined. The areas were fixed by a micrometer grid in the eyepiece of the microscope which was later calibrated against a stage micrometer. The method normally used in haemocytometer counts was employed and every hepatic nucleus or fragment of nucleus which came within the limits was included. The areas counted were selected at random. If, for instance, the area lay partly over a large blood-vessel the hepatic nuclear count for the area was correspondingly diminished and in extreme cases became zero. All such counts were included as contributing to the final result. From the figures of the hepatic nuclear count, the total area counted in each section, and the measured thickness of the section, the corresponding number of hepatic nuclei per cubic centimetre of liver was calculated for each section. This concentration of hepatic nuclei may be assumed to represent the concentration of hepatic cells, since according to Wilson & Leduc (1948) binuclarity is rare in hepatic cells in the prenatal period. In the calculation of the concentration, the Floderus–Abercrombie correction was employed to take account of the effect of the size of the nucleus on the value of concentration obtained (Floderus, 1944; Abercrombie, 1946). To apply this correction the average width of 20 nuclei was measured, 10 measured in the direction of compression of the section and 10 at right angles to the direction of compression. Nuclei were chosen at random but measurements were made only on those whose greatest diameters lay within the section, as seen by focusing up and down. A value for the concentration of hepatic cells was thus obtained for each of six sections of each liver. The mean of these values was taken as the average concentration of hepatic cells throughout the liver. Since the counts of hepatic cells were made at random irrespective of the presence of other tissues in the field, the value of the average concentration of hepatic cells applies to the volume of the liver as a whole with all its component tissues including blood-vessels.

The scatter of the six sectional concentration values was used as an index, both of the error in the method employed and of the real variation of cell concentration in the tissue. The coefficients of variation of the averages of the six sectional concentration values for each liver ranged from 1·7 to 9·8 per cent. with an average value of 5·1 per cent.

For example, the calculations for foetus No. 37 were performed as in the Table on p. 100.

The value of the average cell concentration was corrected for shrinkage of the block in the processes of preparation by applying the volume shrinkage factor previously determined. The term, 'section compression', as normally used
implies rather a distortion than a true volume compression of the tissue, which seems unlikely in view of the incompressibility of the paraffin embedding medium. Experiment indicated that 'section compression' does not change section volume and thus does not affect the observed cell concentration.

Total area counted per section (two fields) = \(3.50 \times 10^4\) sq. \(\mu\) 
Average nuclear diameter = \(5.9\ \mu\)

<table>
<thead>
<tr>
<th>Sect. No.</th>
<th>Total count</th>
<th>Thickness (\mu)</th>
<th>Concentration of hepatic cells (after Floderus-Abercrombie correction)</th>
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</thead>
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<tr>
<td>1</td>
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<td>10.7</td>
<td>(1.72 \times 10^8)/c.c.</td>
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<tr>
<td>2</td>
<td>92</td>
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<td>(1.60 \times 10^8)/c.c.</td>
</tr>
<tr>
<td>3</td>
<td>105</td>
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<td>(1.78 \times 10^8)/c.c.</td>
</tr>
<tr>
<td>4</td>
<td>93</td>
<td>10.5</td>
<td>(1.60 \times 10^8)/c.c.</td>
</tr>
<tr>
<td>5</td>
<td>93</td>
<td>10.2</td>
<td>(1.65 \times 10^8)/c.c.</td>
</tr>
<tr>
<td>6</td>
<td>68</td>
<td>10.5</td>
<td>(1.18 \times 10^8)/c.c.</td>
</tr>
</tbody>
</table>

Average concentration of hepatic cells = \(1.59 \times 10^8\)/c.c.
Standard deviation of average of concentration values = \(0.086 \times 10^8\)/c.c.
Coefficient of variation = 5.4%

(Note—In Section 6 the presence of a blood-vessel in the field counted caused a low count and therefore a low figure for concentration.)

The average concentration of hepatic cells in each liver was multiplied by the volume of the liver, calculated from the weight using the modal value of the specific gravity, 1.045. This was found to be practically constant in livers of all stages of development; deviations from the modal value were randomly distributed and were attributed to errors in measurement. The product of concentration and volume thus obtained was taken as an estimate of the total number of hepatic cells in each liver.

The error involved in this estimate was composed of the errors in the average concentration and in the shrinkage factor used to correct it, the error in the determination of weight being negligible in comparison. The coefficients of variation of the shrinkage factors ranged from 2.4 to 5.6 per cent. These, when combined with the coefficients of variation of the cell concentrations already given, resulted in values of the error in the estimate of total hepatic cell population ranging from 3.0 to 10.2 per cent. with an average value of 6.2 per cent.

The standard error of estimate indicated by the scatter diagram of relative hepatic cell population (see below) plotted against foetal age is approximately \(1.0 \times 10^8/100\) g. of body-weight which gives a coefficient of variation of estimate between 10 and 17 per cent. Since this includes the effects of individual variation along with those of error, the two different methods of computing the error lead to compatible results.

The relative hepatic cell population, that is, the number of hepatic cells present for every 100 g. of body-weight, was then calculated thus:
relative hepatic cell population =
\[
\frac{\text{total hepatic cell population}}{\text{body-weight in grammes}} \times 100.
\]

This quantity is comparable with the relative liver-weight as may be seen from the formula
\[
\text{relative liver-weight} = \frac{\text{weight of liver in grammes}}{\text{body-weight in grammes}} \times 100.
\]

But the relative hepatic cell population expresses the proportion of true hepatic tissue in the body more accurately than the relative liver-weight since the latter takes account of both hepatic and non-hepatic tissue in the liver.

RESULTS

The results are shown in Table 1. Text-fig. 1 shows the relative liver-weight plotted against the age of the foetus. At the 35th day of development the liver forms 11 per cent. of the body-weight, while at term (150 days) it forms only between 3 and 4 per cent. This decline confirms the results of previous workers (Carlyle, 1945; Wallace, 1945). Text-fig. 2 shows the relative hepatic cell population plotted against age. At the 35th day there are on average \(16 \times 10^8\) hepatic cells present for every 100 g. of body-weight; at term only \(6 \times 10^8/100\) g. of body-weight. It is thus evident that the diminution shown by the relative liver-weight
## Table 1

<table>
<thead>
<tr>
<th>Forehead-rump, length (mm.)</th>
<th>Estimated age (days)</th>
<th>Body-weight (g.)</th>
<th>Liver-weight (g.)</th>
<th>Average concentration of hepatic cells in fixed liver (× 10^6/c.c.)</th>
<th>Total hepatic cell population (× 10⁶)</th>
<th>Coefficient of variation of population estimate (%)</th>
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<tr>
<td>1</td>
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is not due solely to a decrease in the amount of haemopoietic tissue in the liver but that in the course of development there is a real diminution in the proportion of true hepatic tissue present in the foetus. In fact the parallel diminution of cell number and liver-weight is due to the comparative constancy of the concentration of hepatic cells in the liver. Text-fig. 3 shows the average concentration of hepatic cells in the fixed liver (the shrinkage factor having been applied) plotted against the age. There is no statistically significant difference ($0.25 > P > 0.1$)
between the concentrations at the beginning and end of the period of development investigated although there is a fall in concentration in the intervening period which is significant ($P < 0.001$). The place of the diminishing haemopoietic tissue appears to be fully taken up, and more, by an expansion of the sinusoids and possibly also by an increase in the collagen content. As in the regenerating liver (Harkness, 1952) the collagen content of the developing liver may lag behind the parenchyme initially before rising to its definitive value. The increase observed in the firmness of the liver substance as development proceeds supports this suggestion. There may, of course, also be some enlargement of the hepatic cells themselves. This does not, however, appear to be the most important factor in supplanting the haemopoietic tissue. An attempt to measure the changes in size of the individual hepatic cell is now being made.

The scatter of points in Text-figs. 1 and 2 may be compared. That in Text-fig. 1 is almost entirely due to the variation of the individual foetuses since the error involved in weighing the liver is comparatively small. The degree of scatter is nevertheless high. One point is particularly far away from the rest, representing a foetus (serial No. 34) aged 83 days whose relative liver-weight is exceptionally high—11.8 per cent. Histological examination of the liver of this foetus shows intense congestion of the sinusoids with blood which accounts for the high value. In general, similar temporary irregularities may produce the considerable variation which occurs in the relative liver-weight. On the other hand, the scatter in Text-fig. 2 is due to a combination of individual variation and experimental errors. The scatter is, however, hardly greater than that in Text-fig. 1. This may be accounted for by the fact that although the estimate of total hepatic cell population is subject to error, it varies much less from one individual to another since it is not affected by temporary difference in physiological conditions.

**DISCUSSION**

It has been seen from Text-fig. 2 that the relative hepatic cell population declines to approximately one-third of its initial value between the 35th day of development and term. Such inconstant relative growth has been variously explained; it was called heteronomous growth by Schmalhausen (1927) and heterogonic growth by Huxley (1932). In the present work an endeavour was made to elucidate the underlying cause of the decrease in the relative hepatic cell population by searching for some other factor which declines in a similar manner. Thus the relative hepatic cell population came to be compared with the specific growth-rate of the foetal body.

The specific growth-rate was obtained from data of body-weight ($W$) and age ($T$) by a simple graphical method. Logarithms were taken of both $W$ and $T$ so as to rectify the resulting curve as far as possible. A 'best line' was drawn through the data and the gradient of this measured directly at short intervals along the log $T$ axis. Each value of the gradient thus measured was then plotted against the mid-value of the log $T$ interval from which it was derived. This resulted in a
practically linear relationship between the gradient $\frac{\delta \log W}{\delta \log T}$ and $\log T$, whence the following equation was derived:

$$\frac{\delta \log W}{\delta \log T} = 20.12 - 7.95 \log T \approx \frac{d \log W}{d \log T} = \frac{T}{W} \frac{dW}{dT}.$$  

Thus

$$\text{specific growth-rate} = \frac{1}{W} \cdot \frac{dW}{dT} = \frac{20.12 - 7.95 \log T}{T}.$$  

The values for the specific growth-rate so obtained are in good agreement with those which result from the same data by the formula of Schmalhausen (1927):

$$\frac{1}{W} \frac{dW}{dT} = \log W_2 - \log W_1.$$  

The specific growth-rate of each foetus was thus calculated and the resulting relationship to the age is shown graphically in Text-fig. 4. The specific growth-rate falls from 22.4 per cent. per day at the 35th day to 1.7 per cent. per day at term.

It may be observed that the real specific growth-rate of an individual foetus can only properly be obtained from successive observations of its weight at intervals during its development. Estimates derived by any method from the weights of a series of foetuses are at best only approximations. The fact that the ages were estimated indirectly from the forehead-rump length may be expected
to introduce an error into the estimate of the specific growth-rate but this hardly affects the accuracy of the final result since, as pointed out, error is inevitably already present.

**Correlation between relative hepatic cell population and specific growth-rate**

The form of the curve of the specific growth-rate shown in Text-fig. 4 is seen to be very similar to that of the relative hepatic cell population in Text-fig. 2.

![Text-fig. 5](image)

This suggests a relationship between these two quantities. The presence of such a relationship may be further investigated by plotting the two quantities against one another. The result of this is shown in Text-fig. 5. This reveals a linear correlation between the relative hepatic cell population and the specific growth-rate between the 35th day of development and term. The correlation is expressed fairly accurately by the equation,

\[ N = 0.5 \times 10^8 R + 5 \times 10^8, \]

where \( N \) is the relative hepatic cell population per 100 g. of body-weight and \( R \) is the specific growth-rate in per cent. per day. A straight line expressing this equation has been drawn through the data of Text-fig. 5.

This proportionality between the relative hepatic cell population and the specific growth-rate suggests that a definite number of hepatic cells is required to maintain a given rate of growth, i.e. that each 100 g. of foetal tissue requires the activity of \( 0.5 \times 10^8 \) hepatic cells for every unit of 1 per cent. per day in its rate of growth. The equation constant, \( 5 \times 10^8 /100 \) g. of body-weight, may be taken to be the number of hepatic cells which is required to maintain the normal metabolic processes of a given 100 g. of foetal tissue apart from its growth.
It must, of course, be emphasized that many natural rates decline with increasing age and that the correlation of the relative hepatic cell population with the specific growth-rate does not prove a direct functional connexion between them. It is no more than consistent with the presumption that such a connexion exists.

The assumption of an anabolic function of the liver in growth is not confirmed by any conclusive evidence so far available. Biochemical knowledge at present relates more to dissimilative than to synthetic processes. It is, however, known that synthesis of plasma proteins, fibrinogen, and prothrombin takes place in the liver and it seems not unreasonable to assign to it an important place in the synthetic mechanisms of growth. It may, of course, be assumed that before the appearance of the liver, the comparatively undifferentiated somatic cells are capable of conducting the growth syntheses by themselves. The variation of the number of hepatic cells in response to functional demand may be likened to the hypertrophy of muscle in response to work, or to wasting following disuse.

An excellent illustration of the effect of functional demand in controlling organ size is provided by the work of Walter & Addis (1939). In experiments in which the metabolic- and growth-rates of young rats were raised or lowered by thyroid administration or thyroidectomy, they found that thyroid administration produced a rise and thyroidectomy a fall in the relative weights of the heart, kidney, and liver. The failure of Evans, Simpson, & Li (1948) to find a similar effect after injection of pituitary growth hormone into rats may be due to the fact that the increase they produced in the specific growth-rate was only 0.12 per cent. per day.

Comparison of the total amount of hepatic substance with the functional demands made upon it may be criticized on the ground that the adult liver has a large functional reserve, e.g. that 80 per cent. of it may be removed without any obvious impairment of hepatic function (Bollmann & Mann, 1936). However, there is no reason to suppose that the foetal liver has a similar reserve; indeed, the investigation of Findlay, Higgins, & Stanier (1947) into the cause of icterus neonatorum suggests that the foetal liver has little or no reserve and that the extra demands made on it immediately after birth result in temporary hepatic insufficiency. In any case, as pointed out by Abercrombie (1955), since even the adult liver regenerates rapidly to its normal size after partial hepatectomy, there must be at least one function for which the liver has no reserve. From experiments in parabiosis and tissue culture it seems likely that this function is the utilization or production of some substance whose blood-level controls the size of the liver (Bucher, Scott, & Aub, 1951; Glinos & Gey, 1952). The regulation of the amount of hepatic tissue in the foetus may well be attributed to a similar humoral mechanism.

One fact inconsistent with the above theory may be noted. According to the work of Schmalhausen (1926) on the chick and of Kaufman (1930) on the chick and pigeon, the relative liver-weight increases during embryonic life while the specific growth-rate as usual declines. This effect may of course be due to the
growth of non-hepatic tissue in the liver, and it will be necessary to discover the
behaviour of the true hepatic tissue in the chick liver. In the dog-fish the relative
liver-weight declines in the prenatal period as in mammals (Kearney, 1914).

Latimer (1948) has shown that in the foetal cat nine organs reach their maxi-
mum relative weight at the close of the embryonic stage and thereafter undergo
a relative decline; these are the heart, liver, pituitary, thyroid, brain, spinal cord,
eyeballs, suprarenals, and ovaries. Of these organs the brain, spinal cord, and
eyeballs have no obvious connexion with bodily growth and their decline must
be put down to other factors. The ovaries are subject to control by the pituitary
gonadotrophic hormones. The relative decline of the heart, pituitary, thyroid,
and suprarenals as well as of the liver may, however, be related to the concurrent
decrease in the specific growth-rate. This question requires further investigation.

There is one important consequence of this theory of liver growth which pro-
vides an opportunity of testing it. The approach which has been used to the
problem of heterogonic liver growth is a physiological one, since it has been
assumed that the amount of hepatic functional capacity present in the foetus at
any time is determined by the functional demands made upon it. Thus the decline
in the relative hepatic cell population may be due either (1) to an increase in the
functional capacity of the individual hepatic cell, functional demand remaining
constant, or (2) to a fall in functional demand. If the decline is to be accounted
for mainly by a fall in functional demand due to decrease in the specific growth-
rate, it must follow that the individual hepatic cell of the sheep foetus does not
change its metabolic activity significantly between the 35th day of development
and term, i.e. that it is functionally almost fully mature before the 35th day. An
attempt is now being made to test this conclusion.

**SUMMARY**

1. A method is described for estimating the total population of hepatic cells
in the liver of a sheep foetus.
2. Use of this method has shown that during development there is a real
decrease in the relative number of hepatic cells in the sheep foetus.
3. The relative number of hepatic cells in the foetus is directly proportional to
the specific growth-rate of the foetal body.
4. It is suggested that the relative number of hepatic cells in the foetus is
determined by functional requirements, partly to perform normal metabolic
processes and partly to maintain growth.

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