Cell population kinetics of erythroid tissue in the liver of foetal mice

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SUMMARY

Measurements have been made of the relative and total numbers of the various types of erythroid cells in the livers of random-bred Swiss foetal mice. It has been shown that the results can be interpreted in terms of two waves of erythropoiesis passing through the liver, one coinciding approximately with the erythropoietin sensitive phase of hepatic erythropoiesis, the other with the insensitive phase.

The mitotic index and thymidine labelling index of early erythroid cells do not change during the period studied, but the labelling index of the late cell types decreases from 30% at 13 days to 15% at 18 days. This decrease has been interpreted as an increase in the proportion of non-dividing polychromatic erythroblasts.

The cell cycle time of early and late erythroid cells in foetal livers has been determined at 13 and 16 days of age. The cycle time of the early cells is about 5 h at both stages of development, but that of late (polychromatic) erythroblasts decreases from 8 h at 13 days to 5-6 h at 16 days.

It has been shown that the results presented are consistent with the change in the rate of increase of hepatogenic red cells which is observed at about the 15th day of foetal life.

INTRODUCTION

Erythropoiesis in mammalian foetuses occurs first in the yolk-sac, followed by the liver, spleen and bone marrow. In most species the bone marrow is the only active site of erythropoiesis in adult life, but under conditions of increased demand for erythrocytes red cell production may be re-initiated in the spleen and occasionally in the liver.

During the hepatic phase of foetal erythropoiesis, which in the mouse occupies the last 7 or 8 days before birth, the number of hepatogenic red cells (erythrocytes and reticulocytes) in the circulation increases in two very distinct exponential phases, with doubling times of 8 and 48 h respectively (Paul, Conkie & Freshney, 1969). The transition occurs at the 15th day of foetal life. This sudden transition from a fast growing population to a relatively slow growing one must reflect changes in the precursor population in the liver, unless there is a sudden increase in the rate of destruction of hepatogenic red cells.

During the period of rapid increase in the number of hepatogenic red cells, erythroblasts taken from mouse foetal livers can be stimulated to increase their

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in vitro rate of haem synthesis by addition of the hormone erythropoietin to the cultures, but during the period corresponding to that of slower growth the cultures cannot be stimulated (Cole, Hunter & Paul, 1968). The transition is quite sharp, and occurs on the 14th or 15th day of foetal life. In adult mammals erythropoietin is necessary for the differentiation of primitive cells into haem-synthesizing cells, and when its level is increased above the normal level during haemolytic or haemorrhagic anaemia the number of cells differentiating per unit time increases (Alpen & Cranmore, 1959; Tarbutt, 1969), and the cell cycle time of the maturing erythroblasts is decreased (Hanna, Tarbutt & Lamerton, 1969).

The present paper describes an investigation of the cell population kinetics of the erythroid system during the hepatic phase of foetal erythropoiesis. Estimates have been made of the numbers of the various types of erythroid precursor as a function of foetal age, as well as the mitotic index and thymidine labelling index of these cells, and the duration of the cell cycle at two different stages of foetal development, the first stage corresponding to the period of rapid increase in the number of hepatogenic red blood cells, the second to the period of slow increase. The two stages of foetal development at which the cell cycle investigations were carried out also correspond to the periods of sensitivity and nonsensitivity to erythropoietin respectively.

**MATERIALS AND METHODS**

Mouse foetuses were obtained from random bred Swiss albino mice (Porton strain) mated after induction of ovulation with 'Gestyl' and 'Pregnyl' (Organon Ltd, Morden, Surrey, England). Foetal age was calculated from the morning after pairing of the males and females.

Foetal livers were removed whole in ice-cold Hanks's balanced salt solution, and disaggregated in 10 ml of Hanks's solution by drawing the tissue repeatedly through a thin polythene tube attached to a syringe. The nucleated cell concentration of a sample of the solution was measured on a Coulter Counter Model D at threshold 50, aperture current 2. Cross checks with haemocytometer counts agreed well at all stages of foetal development. Smear preparations were made from the disaggregated tissue by centrifuging the cells out of suspension, resuspending them in a small drop of mouse serum, and smearing this suspension directly onto clean glass slides. The smears were air-dried, and fixed in methanol for 10 min.

Autoradiographs were prepared from liver smears made from foetuses taken from pregnant females which had previously been injected intraperitoneally with tritiated thymidine (1–2μ Ci/g). After fixation in methanol, the smears were washed for 1 h in running water and then coated with Ilford K5 nuclear emulsion diluted 1:1 with distilled water. After a suitable exposure time (4–12 weeks) the slides were developed with Kodak D19 developer, and fixed in
Johnson's Fixsol diluted 1:7 with distilled water. After fixation the autoradiographs were washed for 2 h in running water, and then soaked for 1 h in a buffer solution at pH 7.0. When the buffered preparations were dry they were stained with Lepehne's stain for 5 min followed by Giemsa stain diluted 1:9 with buffered water (pH 7.0). The autoradiographs were very slightly overstained and the excess stain removed by soaking the preparations in buffer for about 10 min. All preparations were examined at a magnification of \(\times 1000\).

**RESULTS**

**A. Total number of nucleated cells per liver**

Fig. 1 shows the total number of nucleated cells per liver, plotted against foetal age. The number of cells increases steadily until the 18th day, when it reaches a peak of approximately \(7 \times 10^7\) cells, before falling to a somewhat lower value just before birth. These results are in general agreement with those published by Silini, Pozzi & Pons (1967) and Paul et al. (1969).

**B. Number of nucleated erythroblasts per liver**

The number of nucleated erythroblasts expressed as a percentage of the total number of nucleated cells was measured by examining 1000 nucleated cells on each of several smears, care being taken to ensure that the cells were a representa-
tive sample of the whole population. This was achieved by counting equally spaced fields in each of five strips of the smear, the strips being equidistant from each other. The results are shown in Fig. 2A. The percentage of erythroid cells increases very rapidly between 12 and 13 days of age, and then decreases steadily to about 20% at birth. These results are similar to those presented by Paul et al. (1969) except that these authors reported a higher percentage of erythroid cells at 12 days than that observed in the present experiments, and approximately 10% more erythroblasts at all other points. It should be noted that Paul et al. (1969) included reticulocytes and erythrocytes in their results, so that they have to be modified somewhat before they can be compared with the present results.

Fig. 3A shows the product of the total nucleated cell count (Fig. 1) and the percentage of erythroblasts (Fig. 2A), i.e. the total number of erythroblasts per liver. The results are similar in general detail to those presented by Paul et al. (1969), but the peak observed by those authors was about 25% higher and somewhat broader than that shown in Fig. 3.

C. Number of reticulocytes per liver

The number of hepatic reticulocytes compared to the number of erythroblasts is shown in Fig. 2B, and is more or less constant between 12 and 14 days of age, after which it rises linearly, reaching a value of 0.9 just before birth. From Figs. 2B and 3A it is possible to calculate the total number of reticulocytes per liver, as shown in Fig. 3B.

D. Number of erythroblasts at each stage of maturity

The nucleated erythroblasts were classified according to maturity into the following four categories: (a) pro-erythroblasts, the least mature cells that can be identified morphologically as erythroid precursors; (b) basophilic erythroblasts; (c) polychromatic erythroblasts; (d) orthochromatic erythroblasts. In Fig. 4 the relative proportions of these cells are plotted against foetal age. The point at 11 days represents only one sample, obtained by pooling livers from several animals; this was necessary because the livers were extremely small at this stage, and individually contained too few cells to make smearable suspensions. At day 11 practically all of the erythroblasts were early cells, 75% being pro-erythroblasts, 22% basophilic erythroblasts and only 3% polychromatic erythroblasts. No orthochromatic erythroblasts were observed. This suggests that hepatic erythropoiesis starts up independently of yolk-sac erythropoiesis, i.e. there is no mass migration of cells from the yolk-sac to the liver, unless it is a migration only of the very early cells. At 15 days there is a pronounced peak in the proportion of basophilic erythroblasts, with corresponding troughs in the curves for polychromatic and orthochromatic erythroblasts. These features will be discussed in some detail later.

The total number of each type of erythroblast (Fig. 5) was obtained from the
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The product of the differential count (Fig. 4) and the total number of nucleated erythroblasts (Fig. 3A). The number of pro-erythroblasts reaches its maximum value at about 13 days, the number of basophilic erythroblasts peaks at 15 days, the polychromatics at 16 days and the orthochromatics also at 16 days. The curves for basophilic, polychromat and orthochromatic erythroblasts all tend to level off somewhat before rising to their respective peaks; this will be discussed later.

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**E. Mitotic indices of erythroblasts**

As relatively few mitoses were observed in the 1000 nucleated cells examined for the differential counts, no attempt was made to classify the mitotic cells according to maturity, but instead an overall mitotic index was calculated for erythroblasts of all types except orthochromatic erythroblasts, which do not divide. This is shown in Fig. 6. The results show considerable scatter, but there is no evidence of any significant change with foetal age. Seventy per cent of the points lie within the range 3½ to 5½%, the mean being at 4½%.
Fig. 4. Relative proportions of erythroblasts at different stages of maturity. •—•, Pro-erythroblasts; +—+, basophilic erythroblasts; □—□, polychromatic erythroblasts; △—△, orthochromatic erythroblasts.

Fig. 5. Total numbers of erythroblasts at different stages of maturity. •—•, Pro-erythroblasts; +—+, basophilic erythroblasts; □—□, polychromatic erythroblasts; △—△, orthochromatic erythroblasts.

Fig. 6. The mitotic index of hepatic erythroblasts, plotted against foetal age. Seventy per cent of the points lie within the limits shown at $3\frac{1}{2}$ and $5\frac{1}{2}$%. 
In order to determine the proportion of erythroblasts synthesizing DNA, pregnant females were injected with $1\mu$Ci/g of tritiated thymidine ($^3$H-TdR), the foetuses removed $\frac{1}{2}$–1 h later, and autoradiographs prepared as described above. Fig. 7A shows the overall labelling index of erythroblasts of all types except the non-proliferating orthochromatic erythroblasts. The labelling index decreases from about 60% at 13 days to about 45% at 18 days. Fig. 7B shows that this decrease is due to a decrease in the labelling index of polychromatic erythroblasts, from about 30% at 13 days to 15% at 18 days. The labelling index of basophilic erythroblasts does not change significantly throughout the period studied, and the limited data available for pro-erythroblasts (not shown on the diagram, since too few cells were examined from the older animals) suggest that in these early cells the labelling index also does not change with age.
G. The cell cycle of erythroid cells in 13- and 16-day foetal mice

The cell cycle time of erythroid cells was measured by the technique of labelled mitoses (Quastler & Sherman, 1959), in which the proportion of mitotic figures labelled with tritiated thymidine is plotted against time after injection of the label. In a population in which every cell has the same cell cycle the labelled mitoses curve should have the form shown in Fig. 8 A, from which it can be seen that the duration of every phase of the cell cycle can be estimated. Any ‘spread’ in the cell cycle will have the effect of damping the curve, as shown in Fig. 8 B,

Fig. 8. Theoretical labelled mitoses curves. (A) All cells having the same cell cycle. (B) The effect of spread in the cell cycle. $t_c$ = cell cycle time, $S$ = duration of DNA synthesis, $G_2$ = duration of ‘G_2’ period, $G_1$ = duration of ‘G_1’ period.
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in which case approximate estimates for the mean values of the cell cycle parameters can be obtained.

Thirteen- and sixteen-day foetuses were chosen for this part of the study because they are representative of the phases of rapid and relatively slow increase in hepatogenic red cells respectively, and they also correspond to the erythropoietin sensitive and non-sensitive phases of hepatic erythropoiesis.

The percentage of labelled pro- and basophilic erythroblast mitoses in 13-day livers is shown in Fig. 9A. The results shown in Fig. 9B are similar to those in Fig. 9A, except that 'lightly' labelled mitoses, i.e. mitoses labelled with 10 grains or less, have been included with the unlabelled mitoses. It will be shown later that this procedure serves to improve the accuracy of the cell cycle time measurement, but leads to a slight underestimate of $S$ and a corresponding overestimate of $G_1$. The cell cycle time can be read directly from Fig. 9B as the interval between successive peaks, and is approximately 5.6 h. This cycle time is, as far as the authors are aware, shorter than any cycle time so far reported for any mammalian cell. The $G_2$ period is about 0.7 h, and a rough estimate of $4.5 + 0.7 = 5.2$ h for the $S$ period can be obtained from the width of the first peak in Fig. 9A (but not Fig. 9B, which will give an underestimate). This leaves $5.6 - (4.5 + 0.7) = 0.4$ h for the $G_1$ period.

The labelled mitoses curve for 13-day polychromatic erythroblasts (Fig. 10A) shows that the cell cycle time for these cells is considerably longer than that of
the earlier cell types. This is confirmed in Fig. 10B, in which lightly labelled mitoses (i.e. labelled with 10 grains or less) are included with the unlabelled mitoses; this curve gives an estimate of approximately 8.0 h for the cell cycle time. The $G_2$ period is about 1 h, $S$ about $5\frac{1}{2}$ h and $G_1$ about $1\frac{1}{2}$ h (Fig. 10A).

The labelled mitoses curves for pro- and basophilic erythroblasts in 16-day livers is shown in Fig. 11. The inclusion of ‘lightly labelled’ mitoses with the unlabelled mitoses did not change this curve significantly, since very few lightly labelled cells were observed in this experiment. The cell cycle time ($t_c$), as measured by the interval between the two peaks, is about $5\frac{1}{2}$ h, which is similar to that of the corresponding cells in 13-day livers. The $G_2$ period ($tG_2$) lasts for about 1 h, and the duration of DNA synthesis ($t_s$) is $4-4\frac{1}{2}$ h. Thus the $G_1$ period ($tG_1$) is at most $(5.5 - 4.0 - 1.0) = 0.5$ h. These figures are all very similar to the estimates obtained for the cell cycle parameters of pro- and basophilic erythroblasts in 13-day foetal livers ($t_a = 5.6$ h, $t_s = 4.5$ h, $tG_2 = 0.7$ h, $tG_1 = 0.4$ h).

The labelled mitoses curve for polychromatic erythroblasts in 16-day foetal livers is shown in Fig. 12. The first peak is quite well defined, but there is a lot of spread in the points making up the second peak, which is rather difficult to define. The interval between the two peaks (cell cycle time) cannot be estimated very accurately, but is probably between 5 and 6 h. This is similar to that found for basophilic erythroblasts at 16 days. The peaks in the curve for polychromatic
Erythroblasts (Fig. 12) can be made to coincide with those for basophilic erythroblasts (Fig. 11) by shifting the origin in Fig. 12 about 1 h to the left. This suggests that the cell cycle time is about the same for both basophilic and polychromatric erythroblasts at 16 days, but that $G_2$ is longer in the polychromatric erythroblasts, and $G_1$ correspondingly shorter. A similar finding has been reported for bone marrow cells in normal, bled and phenylhydrazine-treated rats (Hanna et al. 1969). If Fig. 12 is compared with Fig. 10, it will be seen that if the first peaks are made to coincide by shifting the origin, then the second peak of Fig. 12 practically coincides with the trough in Fig. 10. This again suggests that the cell cycle time of 16-day polychromatric erythroblasts is shorter than the 8 h estimated for these cells at 13 days. The $G_2$ period can be estimated at $1\frac{1}{2}$ h, the $S$ period lasts for perhaps 4 h, and $G_1$ is probably less than $\frac{1}{2}$ h.
DISCUSSION

The results presented in this paper for the total number of nucleated cells per liver (Fig. 1) are in general agreement with those reported by Silini et al. (1967) and Paul et al. (1969). The erythroid differential counts (Fig. 4) differ somewhat from those reported by Paul et al. (1969), especially at 15 days when these authors did not observe the peak in the basophilic erythroblast curve. Consequently, there is a serious discrepancy in the estimates for the total numbers of erythroid cells at different stages of maturity; for example, our results show a considerable decrease in the total number of basophilic erythroblasts between 15 and 18 days, whereas Paul et al. (1969) found a slight increase over the same period. Part of the discrepancy could be due to a difference in sample preparation (Paul et al. used a cytocentrifuge), but is more likely to be due to the difficulties involved in cell identification. Our results for the total number of
erythroblasts per liver also differ slightly from those of Paul et al. (1969), who reported a peak value some 25% higher than that shown in Fig. 3.

It is tempting to look for two distinct ‘waves’ of erythroid cells passing through the liver during foetal life, one corresponding to the erythropoietin-sensitive phase of erythropoiesis, the other to the non-sensitive phase. The curves for basophilic, polychromatic and orthochromatic erythroblasts all tend to level off somewhat before rising to their respective peaks, and this could in fact be interpreted as meaning that each curve is really a compound of two different ‘waves’ of cells passing through each stage, as shown in Fig. 13. Whether or not the two components do indeed represent different ‘phases’ of erythropoiesis is open to debate, but it is significant that the second ‘wave’ is initiated some time between the 13th and 14th days of foetal life, which corresponds quite well with the time at which the changeover from erythropoietin-sensitive to non-sensitive erythropoiesis occurs (at 14 days in the animals used). It should be pointed out that the ‘levelling off’ upon which this interpretation depends is due mainly to the existence of the peak in the proportion of basophilic erythroblasts at 15 days,

![Graph](image.png)

Fig. 13. Analysis of the curves shown in Fig. 5 as two separate ‘waves’ of cells passing through the liver. (A) Basophilic erythroblasts. (B) Polychromatic erythroblasts. (C) Orthochromatic erythroblasts.
and to the associated troughs in the curve for polychromatic and orthochromatic erythroblasts (Fig. 5).

The proportion of erythroblasts in mitoses does not appear to change with foetal age (Fig. 6), but the amount of scatter in the data is too great to allow any firm conclusions to be drawn. On the other hand, the proportion of erythroblasts synthesizing DNA (Fig. 7 A) appears to decrease slightly with age, and when this decrease is analysed in terms of different cell types it is seen to be due to a decrease in the labelling index of polychromatic erythroblasts, from about 30% at 13 days to about 15% at 18 days (Fig. 7 B). This decrease could be due to a decrease in the ratio $t_d/t_c$, or to an increase in the proportion of polychromatic erythroblasts that do not divide (the polychromatic erythroblast compartment contains cells in their last division before extrusion of the nucleus). It will be seen later that the ratio $t_d/t_c$ does not change significantly for polychromatics, so that the decrease in labelling index can be attributed to an increase in the proportion of non-dividing cells. The lack of any significant change in the labelling index of the early erythroblasts (all of which divide) makes it unlikely that any change occurs in the cell cycle of these cells, and this is confirmed by the labelled mitoses curves for these cells at 13 and 16 days.

Before going on to discuss the labelled mitoses curves in detail it is necessary to consider the effect of including 'lightly labelled' mitoses with the unlabelled mitoses. Comparison of Fig. 9 A with Fig. 9 B will reveal that this procedure only affects the curve significantly in the regions near to the troughs. The labelled cells observed in mitoses at these intervals must have only just started to synthesize DNA when the label was injected, since these are the last labelled cells to enter mitoses before a second cycle begins. Thus the 'lightly labelled' mitoses correspond only to cells at the beginning of DNA synthesis, and so by regarding these as being 'unlabelled' we are neglecting the early part of DNA synthesis, which will be recorded as part of $G_1$. The estimate of the total duration of the cell cycle is not, of course, altered by this procedure, but the estimate is made more precise because the troughs of the curve are more clearly defined by 'extending' the $G_1$ period. This procedure can only be used when the 'lightly labelled' cells can be shown to occur only at the beginning (or end) of DNA synthesis and is only useful when the sum of $G_1$ and $G_2$ is short.

The cell cycle time of pro- and basophilic erythroblasts is about 5.5 h at both 13 and 16 days, i.e. in both the erythropoietin sensitive and non-sensitive phases of hepatic erythropoiesis. On the other hand, the cell cycle time of the later (polychromatic) erythroblasts decreases from 8 h at 13 days to between 5 and 6 h at 16 days. Cole et al. (1968) have suggested that the change in erythropoietin sensitivity between 14 and 15 days may be due to an increase in the amount of endogenous erythropoietin available to the foetus; thus in the early (pre 14- or 15-day) foetus the cells are maturing and proliferating in the presence of low concentrations of erythropoietin, whereas in the later foetus the concentration of erythropoietin is so high that the cells are already fully stimulated. This interpre-
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tation makes the change in the cell cycle time of polychromatic erythroblasts easy to explain, for it is known that erythroid cells in the bone marrow of adult rats decrease their cycle time when the level of erythropoietin is increased by anaemia (Hanna et al. 1969). The lack of change in the cycle time of the earlier cells may mean that even when the level of erythropoietin is quite low these cells are proliferating near the maximum rate. This interpretation will be discussed again later.

The thymidine labelling index of erythroblasts should be approximately equal to the ratio $t_n/t_e$ if all of the cells divide. For pro- and basophilic erythroblasts this is $4.5/5.5 = 0.82$ at both 13 and 16 days. This compares well with the measured labelling indices of 0.77 at 13 days and 0.74 at 16 days, indicating that all of these cells do divide. The corresponding ratios for polychromatic erythroblasts are $5.5/8.0 = 0.69$ at 13 days and $4.0/5.5 = 0.73$ at 16 days. The measured labelling indices for polychromatics at 13 and 16 days are 0.33 and 0.28 respectively, and hence the proportion of these cells that divide is $0.33/0.69 = 0.48$ at 13 days and $0.28/0.73 = 0.38$ at 16 days. Thus there is a suggestion of a slight decrease in the number of dividing cells between 13 and 16 days; the labelling index falls more rapidly after 16 days (Fig. 7B), so there is probably a further decrease in the proportion of dividing cells after 16 days. This is supported by the morphological appearance of the polychromatic erythroblast compartment, which shows a significant and increasing shift towards more mature polychromatic erythroblasts from the 15th day until birth.

The data presented in this paper are sufficient to predict the approximate numbers of hepatogenic red blood cells (erythrocytes plus reticulocytes) in the mouse foetus as a function of foetal age. Paul et al. (1969) have shown that the number of these cells increases exponentially with a doubling time of 8 h between the 13th and 15th days of foetal life, and with a doubling time of 48 h from the 15th to the 18th day. If the data can predict a similar changeover from a fast to a relatively slow doubling time then it has satisfied a fairly stringent test. It should, however, be pointed out that the calculations which follow are based on several measured parameters, each with its inherent error, so that a changeover as sharp as that described by Paul et al. (1969) should not be expected.

The number of erythroid cells produced per day is given approximately by the formula

$$\text{number produced per day} = 24 \times N^1 \frac{\text{L.I.}}{t_g},$$

where $N^1 =$ number of proliferating erythroblasts present half-way through that day, L.I. = mean thymidine labelling index of proliferating erythroblasts for that day, $t_g =$ mean duration of DNA synthesis. The difference between the observed change in the total number of erythroblasts and the calculated number of erythroid cells produced per day is the number of red cells produced in that day. The results are shown in Table 1.
Between day 12 and 13 (not shown in the table) the increase in erythroblasts is practically equal to the calculated production of erythroid cells, so that during this period the production of red cells is very small. This is confirmed by the results of Paul et al. (1969), who showed that hepatogenic red cells do not begin to appear at all until late in the 12th day. If we accept these authors’ estimate of $3 \times 10^3$ hepatogenic red cells at 13 days, then the number present in any succeeding day can be calculated by adding to this the number produced in the intervals up to the relevant day, i.e. by adding the numbers in the last column in Table 1. The results are shown in Fig. 14. Also shown on this diagram are two log-linear plots, with doubling times of 8 h and 48 h respectively, corresponding to the doubling times observed by Paul et al. (1969). The log-linear plots have been adjusted to coincide with the calculated points at 13 days and 17 days, and it can be seen that the points lie fairly close to these two lines. Thus the data are consistent with the observed bi-phasic pattern of foetal hepatic erythropoiesis, and can predict this pattern quite well.

An important point to emerge from this study is that the significant increase in doubling time of the red cell mass at about the 15th day of foetal life (Paul et al. 1969) is not due to an increase in the cell cycle time of the erythroid precursors. On the contrary, the cell cycle time of the later cell types actually decreases between the 13th and the 16th day. The change observed by Paul et al. (1969) appears instead to be due to a decrease in the absolute number of erythroid cells in the liver, and in the proportion of these cells that are capable of proliferation.

The control mechanisms underlying foetal hepatic erythropoiesis are not at all clear. In the adult mammal erythropoiesis appears to be largely under the control of erythropoietin, but in the foetal or newborn animal this may not be the case. Stohlman et al. (1964) have shown that erythropoiesis in neonatal rats does not cease after hypertransfusion, starvation or nephrectomy, although all

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**Table 1. Comparison of the daily increase in the number of erythroblasts with the number of erythroid cells produced per day**

<table>
<thead>
<tr>
<th>Interval (days)</th>
<th>No. of erythroid cells produced* (N)</th>
<th>Increase (+) or decrease (—) in no. of erythroblasts (N1)</th>
<th>Red cell production (N—N1)</th>
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</thead>
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<tr>
<td>13–14</td>
<td>$29 \times 10^6$</td>
<td>$+8 \times 10^6$</td>
<td>$21 \times 10^6$</td>
</tr>
<tr>
<td>14–15</td>
<td>$43 \times 10^6$</td>
<td>$+6 \times 10^6$</td>
<td>$37 \times 10^6$</td>
</tr>
<tr>
<td>15–16</td>
<td>$52 \times 10^6$</td>
<td>$+1.5 \times 10^6$</td>
<td>$51 \times 10^6$</td>
</tr>
<tr>
<td>16–17</td>
<td>$42 \times 10^6$</td>
<td>$0 \times 10^6$</td>
<td>$42 \times 10^6$</td>
</tr>
<tr>
<td>17–18</td>
<td>$36 \times 10^6$</td>
<td>$-5 \times 10^6$</td>
<td>$41 \times 10^6$</td>
</tr>
<tr>
<td>18–19</td>
<td>$18 \times 10^6$</td>
<td>$-11 \times 10^6$</td>
<td>$29 \times 10^6$</td>
</tr>
</tbody>
</table>

* Based on $t_s = 5$ h on days 13 and 14; $t_s = 4\frac{1}{2}$ h on days 15–19.
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of these procedures abolish erythropoiesis in adult rats. On the other hand erythropoietin has been demonstrated in foetal and neonatal blood from animals of several different species (Halvorsen, 1963), and the rate of haem synthesis in hepatic erythroblasts from early foetuses can be increased in vitro by the addition of erythropoietin (Cole et al. 1968). The number of haemopoietic stem cells in foetal livers continues to increase at least until birth (Silini et al. 1967), but the number of erythroblasts decreases in an orderly manner from the 15th day onwards (Fig. 5). Thus it appears that a factor controlling the transition of primitive cells into pro-erythroblasts is ineffective during this period. In adult mammals the transition from stem cell to pro-erythroblast is not direct, but proceeds via an intermediate cell, which itself is transformed to a pro-erythroblast only in the presence of erythropoietin (Bruce & McCulloch, 1964). If the transition from erythropoietin sensitive cell to pro-erythroblast is blocked in the later foetuses, then it seems likely that this is due to inefficient utilization of erythropoietin during this period. The observation of Cole et al. (1968) that

![Diagram](image_url)

Fig. 14. The calculated number of hepatogenic red blood cells (including reticulocytes) as a function of foetal age. The broken lines represent doubling times ($T_D$) of 8 and 48 h, as observed by Paul et al. (1969).
erythroblasts from foetuses over 14 days of age are not sensitive to exogenous erythropoietin is consistent with the hypothesis that erythropoietin utilization is inefficient in these late foetuses, but if this is the case the reduction in cycle time of polychromatic erythroblasts between 13 and 16 days (Figs. 10, 12) cannot be attributed simply to an increased concentration of erythropoietin, unless it is assumed that proliferation can be stimulated by erythropoietin independently of haem synthesis.

RÉSUMÉ

Cinématiques de la population cellulaire du tissu érythroid dans le foie de souris fœtales

Des mesures des nombres relatif et total des différents types de cellules érythroides ont été effectuées dans les foies de souris fœtales, croisées au hasard. Les résultats montrent qu'il existe deux vagues d'érythropoïèse dans le foie, l'une correspondant approximativement à la phase érythropoïétique sensible de l'érythropoïèse hépatique, l'autre à la phase insensible.

L'index mitotique et l'index de marquage par la thymidine des jeunes cellules érythroides ne changent pas pendant la période étudiée, mais l'index de marquage des cellules âgées décroit de 30 %, à 13 jours, à 15 % à 18 jours. Cette diminution est interprétée comme une augmentation de la proportion en érythroblastes polychromatiques, ne se divisant pas.

La durée du cycle cellulaire des cellules érythroides jeunes et âgées, dans les foies foetaux a été déterminée à l'âge de 13 et de 16 jours. Celle des cellules jeunes est d'environ 5,5 h aux deux stades du développement mais celle des érythroblastes (polychromatiques) âgés, décroit de 8 h, à 13 jours, à 5 ou 6 h, à 16 jours.

Ces résultats peuvent être expliqués par le changement dans le taux d'accroissement des cellules rouges hépatogènes, qui est observé au 15ème jour environ, de la vie foetale.

The authors wish to thank Miss S. L. Smith and Mrs M. J. Reed for their technical assistance. The work described in this paper was supported by a grant from the Medical Research Council.

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(Manuscript received 16 January 1970)