# CHANGES IN THE BONE MARROW AND BLOOD CELLS OF DEVELOPING RABBITS

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PLATES 7 TO 9

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We have studied the cells of the blood and bone marrow of young rabbits, starting with late fetal stages and following the changes that take place at birth and during the first 6 months of life. By that time the rabbit is mature and the different strains of cells in the blood have reached the proportions characteristic of the adult. The changing pattern of the blood cells finds its explanation mainly in changes in the bone marrow.

As is known, the tissues of the so called marrow cavity are not at once associated with the formation of blood cells. Rather the first function of this organ is in relation to the development of the bone. In the rabbit the changes in the original cartilage begin in the center of the shaft of the long bones during the 17th and 18th days of gestation (1). The primary vascular pattern is of large sinusoids which, beginning in the center of the shaft, soon extend to form loops between the lines of the cartilage cells at each epiphyseal line. Between the sinusoids there is a delicate reticulum containing sparsely scattered connective tissue cells embedded in a gelatinous matrix. There are no fat cells. Along the edges of the developing bone and the regressing cartilage there are osteoblasts or multinucleated osteoclasts, the latter formed, as Arey (2, 3) has shown, by the fusion of osteoblasts. The study of bone marrow as an hematopoietic organ is concerned with the method by which this simple structure becomes changed to provide for the development of three different strains of cells, namely, red blood cells, granulocytes, and megalokaryocytes.

TABLE I

*Blood Cells of 115 Rabbits for the First 6 Months of Life*

| Age of rabbits | No. of rabbits | No. of counts | No. of W.B.C. counted in differentials | Red blood cells | Hemoglobin | Color index | White blood cells | Granulocytes | | | Lymphocytes | Monocytes | Myelocytes | Myeloblasts | Clasmatocytes | Megalokaryocytes | Degenerating | Unclassified | Platelets |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Neutrophiles | Basophiles | Eosinophiles | | | | | | | | | |
| | | | | | *per cent* | | | | | | | | | | | | | | |
| 28th fetal day | 7 | 7 | 700 | 2,834,000 | 65 | 1.14 | 960 | 529 | 75 | 17 | 113 | 196 | 21 | 1 | — | — | — | 5 | — |
| 29th fetal day | 8 | 8 R.B.C.<br>7 Hb<br>8 W.B.C. | 800 | 3,513,000 | 68 | 0.98 | 978 | 553 | 125 | 15 | 95 | 176 | 10 | 1 | — | — | 1 | 1 | — |
| 30th fetal day | 1 | 1 | 100 | 2,830,000 | 70 | 1.25 | 800 | 288 | 64 | — | 400 | 48 | — | — | — | — | — | — | — |
| Day of birth | 24 | 24 R.B.C.<br>22 Hb<br>22 W.B.C.<br>18 platelets | 2,200 | 4,016,000 | 76 | 0.94 | 2,525 | 1,799 | 119 | 17 | 333 | 211 | 19 | — | — | 8 | 6 | 11 | 481,669 |
| Rest of 1st wk. | 23 | 33 R.B.C.<br>25 Hb<br>33 W.B.C.<br>18 platelets | 3,300 | 3,766,000 | 68 | 0.91 | 2,840 | 1,473 | 145 | 42 | 542 | 617 | 3 | — | 15 | — | 1 | — | 377,000 |
| 2nd wk. | 19 | 26 R.B.C.<br>20 Hb<br>26 W.B.C. | 2,600 | 4,303,000 | 70 | 0.82 | 2,996 | 1,320 | 154 | 38 | 844 | 617 | 7 | — | 11 | — | 1 | — | — |
| 3rd wk. | 30 | 30 R.B.C.<br>23 Hb<br>30 W.B.C. | 3,000 | 5,399,000 | 74 | 0.69 | 3,943 | 1,623 | 323 | 18 | 1,202 | 735 | — | — | 17 | — | 12 | 9 | — |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 4th wk. | 23 | 23 R.B.C.<br>19 Hb<br>23 W.B.C. | 2,300 | 5,124,000 | 70 | 0.70 | 4,267 | 1,508 | 523 | 32 | 1,521 | 670 | 2 | — | — | — | 8 | — | — |
| 2nd mo. | 35 | 94 R.B.C.<br>81 Hb<br>95 W.B.C. | 9,500 | 5,434,000 | 71 | 0.55 | 4,927 | 2,258 | 405 | 39 | 1,411 | 792 | 4 | — | 11 | — | 3 | 1 | — |
| 3rd mo. | 31 | 85 R.B.C.<br>75 Hb<br>85 W.B.C. | 8,500 | 5,501,000 | 66 | 0.58 | 5,964 | 2,515 | 379 | 46 | 2,173 | 826 | 1 | — | 17 | — | 3 | 1 | — |
| 4th mo. | 21 | 51 R.B.C.<br>41 Hb<br>51 W.B.C. | 5,100 | 5,639,000 | 64 | 0.56 | 6.404 | 2,690 | 469 | 78 | 2,513 | 626 | 2 | — | 11 | — | 9 | 1 | — |
| 5th mo. | 24 | 70 R.B.C.<br>68 Hb<br>70 W.B.C. | 12,400 | 5,602,000 | 73 | 0.65 | 7,924 | 3,305 | 448 | 167 | 3,266 | 721 | 6 | — | 3 | — | 3 | 1 | — |
| 6th mo. | 29 | 100 | 22,600 | 5,751,000 | 79 | 0.68 | 9,829 | 4,152 | 500 | 137 | 4,069 | 954 | 8 | — | 1 | — | 4 | 2 | — |

## *Materials and Methods*

132 rabbits were used in the experiment. They were bred at the Institute and all of each litter were used. Blood counts were made on 115 rabbits and counts of the marrow cells on 49. 32 animals had studies of both blood and marrow cells. Since some of each litter were killed from time to time for the studies of the bone marrow, other rabbits were added for the blood counts of the 5th and 6th months. In the earlier group, the animals were of several different breeds, including a few New Zealand Reds; the animals added for the 5th and 6th months were all New Zealand Reds.

For the fetal stages and the first 2 weeks of life the blood was obtained by heart puncture; after that from an ear vein. The platelets are high in early life and the blood clots with extreme rapidity. It is thus necessary to put the blood from the syringe into a paraffined watch glass and have different people take the blood for the several preparations immediately and simultaneously.

The preparations of the bone marrow were made from three bones, the femur, the tibia, and the humerus. The supravital technique (4–7) was used entirely for the counts of blood cells. This technique has a great advantage in the study of the blood cells of animals since it allows a better discrimination of the monocyte. The granulation of this cell, to be seen in fixed films of human blood, is lacking in the corresponding cell in rabbits, probably correlated with the fact that the monocyte in the blood of rabbits is oxidase-negative. The lack of this granulation makes difficult its discrimination from the lymphocyte in fixed films of rabbits' blood. In the supravital technique the differentiation of the monocyte is made by other characteristics,—the vacuoles, the mitochondria, the surface film, and the type of motility.

In making the differential counts of the blood cells, 100 cells were counted from each animal through the 4th month, as shown in Table I. For the 5th month, 10 of the animals and 22 of the counts were from the original series and hence of 100 cells each; 14 of the rabbits were from the new series, with 48 counts of 200 or 400 each, making a total of 12,400 cells counted. For the 6th month only 7 animals were left from the first series, making 700 cells counted from this group. The other animals, 93 in all, had counts of 200, 400, 500, or in two instances of 1,000 cells, making a total of 22,600 cells. In the entire series for all ages, 73,100 cells were counted. In general our present procedure in making blood counts is to count 100 cells from each of 2 drops of blood and use the average in case the 2 counts correlate. If they do not, to count from 400 to 1,000 cells from fresh preparations.

For the counts of marrow cells, it is our opinion that the supravital technique is also preferable, since the method allows more accurate differentiation and the alteration of cells is minimal. All the counts of the marrow cells recorded in the table and on the graphs were made by this method. In 15 instances duplicate counts of the marrow cells were made from fixed films. The correlation was good in 7 and poor in 8 counts, and it is our opinion that the supravital technique is the

better standard. For the fetal marrow and for that during the first 2 weeks of life, the preparations of the marrow can be made either by sucking the marrow a short distance into a capillary pipette and transferring to the slide, or by squeezing the entire marrow out of the bone after carefully removing muscle, tendon, and periosteum from its surface. In the early stages the marrow is so fluid that it spreads readily with the weight of the coverslip; in later stages, when fat has appeared, a tiny bit of the marrow, placed on a slide, can be spread by slow and gentle pressure on the coverslip.

The counts of marrow cells cannot be as accurate as blood counts since there must be a choice of fields. Only zones in which the cells are in a single layer and slightly separated from each other can be counted; also they must be well stained. For these reasons it is not possible to count systematically across a film and back, as with films of blood. These factors make it advisable to count more cells than for blood and as a result of our studies we think that 5,000 cells should be the minimum for each animal.

The cells of the marrow can also be counted from sections if they meet certain standards. The fixative, as Maximow (1) showed, should be Zenker-formol; the formol should be free from acid. The absence of acid prevents the solution of hemoglobin and of certain granulations. The sections must then be so thin, not more than 4$\mu$, that the cells are all in a single layer, and the staining, Giemsa preferred, such as to give maximum discrimination.

## RESULTS

### *Blood Counts*

The pattern of the blood cells of these young rabbits is shown on Chart 1,[1] from data which are given in Table I. In making the chart, the intervals of time for the fetal period are spaced arbitrarily. Rabbits are born sometime between the 28th and 32nd day after mating. As is shown on Chart 1, the number of red cells in the rabbit's blood is still low in the late fetal days, with a tendency to rise on the day of birth. In the 7 animals of a litter counted on the 28th fetal day, the range was wide, varying from 2,210,000 to 3,290,000 cells, and there was a variation in hemoglobin of 13 points. On the 29th fetal day, the range was less, namely, from 3,205,000 to 3,865,000 cells, with the hemoglobin varying 12 points. All but one in this group were from the same litter and this one had a count near the average, namely, 3,490,000 cells. The drop in red cells, shown on the 30th day of gestation, has probably little significance, since this was a single count taken from one of a litter. The rise on the day of birth was, however, significant, only one animal of the 22 counted falling below 3,000,000 cells. The range was from 2,760,000 to 5,210,000 cells and 16 of the rabbits had counts between 3,400,000 and 4,400,000 red cells.

[1] The figures represented on Charts 1, 2, and 3 are recorded as logarithms so that the relativity of each change is better expressed.

During the rest of the 1st week of life, there was a slight fall in erythrocytes, amounting to 250,000 cells. Of the 33 animals counted, 21 had counts under 4,016,000, the average on the day of birth, but in the group there were 4 animals which had 3 counts during this period, namely, on the 2nd, the 4th, and the 6th days of life. Of these 4, 2 showed a steady rise in the red cells, while one had a fall of 500,000 red cells on the 2nd count, with recovery on the 3rd, and the 4th had a fall of 800,000 cells on the 3rd count. The loss in red cells in these 2 rabbits may well have been due to the fact that for the heart punctures, 3 in number,

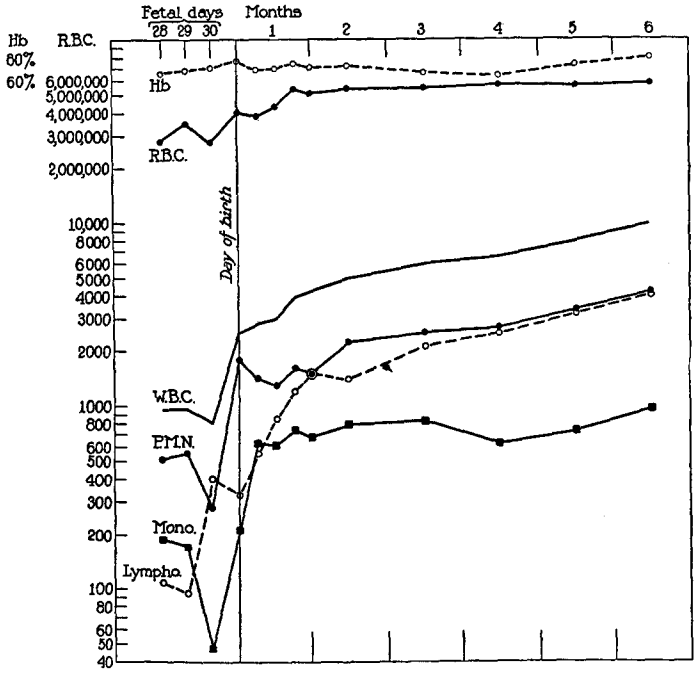


CHART 1. Blood cells of 115 young rabbits from data in Table I.

more blood must be taken than for the later counts from the veins. These two records, therefore, cannot be regarded as a physiological fall in red cells. But if the counts of these 2 animals are omitted from the series, the average of the other 27 counts still shows a fall in red cells, namely, to 3,744,000. The loss, 250,000 cells, is slight, but at least the data establish the fact that the rabbit is unable to produce enough mature red cells to cause a rise in their number in the blood during the 1st week of life.

During the 2nd and 3rd weeks, on the other hand, there was a remarkable rise in red blood cells to a mean level in the 3rd week of life of 5,399,000 cells, which is the established mean of red cells of the adult rabbit. Adequate figures on this

subject have been given by Pearce and Casey (8). For reference, we give Table III.

It will be noticed on Chart 1 that there was a slight drop in red cells in the 4th week, which may indicate a slight reaction after the remarkable activity of the bone marrow toward red cells during the 2nd and the 3rd weeks. The estimation of hemoglobin was made by the colorimetric method with the Newcomer standard. It is known that the colorimetric method has a wide range of error. To limit this error as much as the method permits, all of the readings in the first series were made by one person and the same was true of the second series introduced in the 5th and 6th months. The line of the percentage of the hemoglobin on Chart 1 is, notwithstanding, a remarkably straight line. In column 7 of Table I is given the color index which is shown graphically on Chart 1 by the spread between the lines of the number of red cells and the percentage of hemoglobin. The proportion of hemoglobin per red cell is high in fetal blood, as has recently been shown by Wintrobe and Shumacker (9) and by Kunde *et al.* (10). Chart 1 shows that this condition persists for a time after birth and that the amount of hemoglobin per red cell only gradually approaches the standard of the adult rabbit, which is reached in the 3rd and 4th months of life. The rise in color index for the 5th and 6th months may well be due to a predominance of one breed of rabbit.

The lower half of Chart 1 shows the total numbers of the white blood cell, together with the proportions of three of the strains, namely, the neutrophilic, that is, pseudoeosinophilic,[2] leucocytes, the lymphocytes, and the monocytes. The differential counts are recorded in Table I in terms of total numbers rather than percentages. It will be noted that in contrast to the red cells, the white cells of the blood increase slowly and do not reach the numbers characteristic of the adult animal until the 5th and 6th months. For comparison, our data on the blood cells of the adult rabbit are given in Table IV.

As shown on Chart 1 and Table I, the white blood cells were low in the circulation during the late fetal stages, the average being about 900 cells per c.mm. In the series of 16 counts, the range is from 700 to 1,425 cells; only one count was above 1,175 and 10 of the 16 counts were below 1,000 cells. As with the red cells, there was a sharp rise on the day of birth from the average of 900 to 2,525 cells. On the day of birth there was a wide variation in the limits of the counts, but only one was below 1,200 cells. This was a single count of 300 cells, balanced by 2 high counts of 5,000 and 7,000 cells. Most of the counts, however, fell between the limits of 1,200 and 2,700 cells, with 5 counts of 2,100.

As seen on Chart 1, the rise of the white cells that took place at birth was almost wholly due to neutrophilic leucocytes. This tendency was, however, not sustained during the 1st month, and it was not until the 2nd month that there was initiated the progressive increase which carried them to their full quota in the 5th and 6th months.

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[2]The term neutrophilic is used throughout for the pseudoeosinophilic granulations of rabbit's blood.

TABLE II

*Cells of the Bone Marrow of 49 Rabbits*

| Animal No. | Age | No. of cells counted | Erythroid | | | | | Myeloid | | | | | | | | Accessory | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Normoblasts | Late erythroblasts | Early erythroblasts | Megaloblasts | Total | Neutrophilic leucocytes | Basophilic leucocytes | Eosinophilic leucocytes | Myelocytes C | Myelocytes B | Myelocytes A | Myeloblasts | Total | Primitive cells | Megalokaryocytes | Clasmatocytes | Osteoclasts | Unclassified |
| R 1170 A* | Late fetal† | 750 | 2.80 | 2.13 | 1.33 | — | 6.26 | 0.66 | — | — | 0.40 | 12.66 | 13.86 | 55.49 | 83.07 | 8.13 | — | — | 2.13 | — |
| 4311<br>4312<br>4313<br>4314 | 29th fetal day | 5,000 | 22.74 | 40.56 | 10.60 | 0.18 | 74.08 | 1.84 | 0.64 | 0.08 | 1.74 | 4.68 | 3.02 | 1.74 | 13.74 | 9.88 | 0.06 | 1.98 | 0.26 | — |
| 4302<br>4303<br>4304<br>4305<br>4306 | 30th fetal day | 5,000 | 22.0 | 41.06 | 8.1 | 0.56 | 71.72 | 5.82 | 0.16 | 0.12 | 4.42 | 5.16 | 1.22 | — | 16.90 | 11.0 | — | 0.16 | 0.02 | 0.20 |
| 1658<br>1588<br>1627<br>1640 | Day of birth | 3,550 | 21.07 | 14.56 | 14.00 | 1.27 | 50.90 | 4.90 | 0.39 | 0.14 | 6.02 | 6.00 | 5.07 | 13.43 | 35.95 | 11.35 | 0.47 | 0.92 | — | 0.36 |
| 1779<br>1780<br>1630<br>1424 | 2nd day | 4,219 | 9.05 | 12.51 | 6.94 | 0.87 | 29.37 | 10.76 | 0.85 | 0.14 | 7.70 | 10.64 | 6.99 | 16.09 | 53.17 | 15.71 | 0.11 | 0.63 | 0.26 | 0.71 |
| 4374<br>4375 | 3rd day | 5,000 | 28.32 | 17.30 | 3.54 | 0.01 | 49.17 | 28.34 | 1.70 | 0.42 | 4.32 | 3.76 | 3.52 | 0.40 | 42.46 | 7.02 | 0.08 | 0.90 | 0.04 | 0.32 |
| 1458<br>4376 | 4th day | 6,000 | 39.60 | 34.51 | 4.58 | 0.63 | 79.32 | 6.63 | 0.80 | 0.23 | 2.58 | 1.91 | 1.66 | 0.23 | 14.04 | 5.4 | 0.25 | 0.65 | 0.11 | 0.18 |

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1784<br>4377<br>4396<br>4397 | 5th day | 20,000 | 23.50 | 33.62 | 6.08 | 0.16 | 63.36 | 11.16 | 1.24 | 0.44 | 3.84 | 2.98 | 4.52 | 2.58 | 26.76 | 8.75 | 0.05 | 0.89 | 0.06 | 0.08 |
| 4398<br>4399 | 6th day | 5,000 | 17.48 | 38.22 | 3.38 | — | 59.08 | 15.94 | 1.84 | 0.52 | 3.30 | 4.52 | 6.24 | 3.14 | 35.50 | 3.34 | 0.26 | 1.54 | 0.06 | 0.22 |
| 1286<br>1470 | 7th day | 2,660 | 51.57 | 15.52 | 13.60 | 2.93 | 83.62 | 2.44 | 0.26 | — | 10.37 | 1.80 | 0.78 | — | 15.65 | 0.30 | 0.30 | 0.03 | — | 0.03 |
| 1790<br>1466<br>2046<br>2047 | 2nd wk. | 9,991 | 35.12 | 36.21 | 2.28 | 0.31 | 73.92 | 11.01 | 0.31 | 0.19 | 5.01 | 2.57 | 2.27 | 1.26 | 22.62 | 2.85 | 0.11 | 0.41 | 0.01 | 0.05 |
| 1234<br>1459 | 3rd wk. | 1,894 | 36.85 | 9.13 | 9.13 | 0.58 | 55.69 | 14.46 | 0.21 | 0.05 | 17.84 | 5.38 | 0.84 | 0.10 | 38.88 | 4.75 | 0.26 | 0.36 | — | — |
| 1288<br>1377<br>2048 | 4th wk. | 3,193 | 28.78 | 11.65 | 5.48 | 0.50 | 46.41 | 22.64 | 1.00 | 0.43 | 14.62 | 8.76 | 1.25 | 1.91 | 50.61 | 1.62 | 0.46 | 0.40 | — | 0.43 |
| 1460<br>4307<br>4308<br>4309 | 2nd mo. | 16,559 | 32.85 | 24.40 | 2.95 | 0.03 | 60.23 | 22.56 | 1.26 | 0.67 | 7.19 | 2.86 | 1.97 | 0.27 | 36.78 | 2.49 | 0.19 | 0.22 | — | 0.03 |
| 1306<br>1378 | 3rd mo. | 2,000 | 40.80 | 7.60 | 5.20 | 1.30 | 54.90 | 3.70 | 1.05 | 2.65 | 32.10 | 3.15 | 1.40 | 0.30 | 44.35 | 0.30 | 0.30 | 0.15 | — | — |
| 1226<br>1287 | 4th mo. | 2,000 | 25.05 | 8.05 | 4.25 | 0.75 | 38.10 | 13.45 | 3.00 | 0.85 | 32.85 | 2.60 | 1.35 | 0.70 | 54.80 | 6.15 | 0.80 | 0.15 | — | — |
| 1173<br>4380 | 5th mo. | 6,235 | 29.60 | 21.18 | 0.94 | 0.08 | 51.80 | 31.54 | 1.04 | 1.04 | 7.58 | 2.58 | 2.43 | 0.09 | 46.30 | 1.57 | 0.20 | 0.10 | — | — |

* These are serial numbers of the department covering a term of years.

† The date of mating was not known and hence this record is not shown on Charts 2 and 3.

Quite different from this are the curves for lymphocytes and monocytes. The lymphocytes showed a progressive rise in total numbers from the exceedingly low levels of the late fetal stages to their full quota in the 5th and 6th months of life. This rise was more rapid during the 1st month than thereafter. On the other hand, the monocytes rose rapidly during the 1st week of life to their final quota, which was maintained for the rest of the 5 months. Thus the monocyte is the first cell in the blood stream to reach its normal quota.

TABLE III

*Red Blood Cells in Rabbit*

| | Mean | Mode | Probable error | Standard deviation | Coefficient variation |
|---|---|---|---|---|---|
| | | | | | *per cent* |
| Pearce and Casey (data on 174 normal adult rabbits) | 5,198,000 ± 12,700 | 5,215,000 | 432,748 | 628,250 | 12.09 |
| Data from this laboratory on 62 normal adult rabbits* | 5,366,000 ± 96,406 | 5,400,000 | 440,000 | 660,000 | 12.30 |

* For these data the blood cells of the 62 rabbits were counted 456 times; the mean for each rabbit was obtained and used in calculating the frequency distribution from which the probable error and standard deviation were determined.

TABLE IV

*White Blood Cells in the Rabbit (Data on 62 Animals)**

| | Mean | Mode | Probable error | Standard deviation | Coefficient variation |
|---|---|---|---|---|---|
| | | | | | *per cent* |
| White blood cells | 8,866 | | | | |
| Neutrophilic leucocytes | 3,727 (42.03%) | 3,000 | 1,158 | 1,720 | 31 |
| Basophilic " | 715 (8.06%) | 500 | 342 | 508 | 47 |
| Eosinophilic " | 110 (1.24%) | — | — | — | — |
| Lymphocytes | 3,595 (40.54%) | 3,750 | 952 | 1,422 | 37 |
| Monocytes | 719 (8.10%) | 500 | 356 | 528 | 49 |

* See also the figures of Pearce and Casey (8).

Table I also gives the data for basophiles and eosinophiles. The basophiles rose steadily for the 1st month and then remained at a nearly uniform level for the next 5 months, but they did not reach, during this period, the level previously determined as characteristic of the adult rabbit. Our figures are 715 basophilic leucocytes per c.mm. (Table IV) and those of Pearce and Casey, 950 (8). The eosinophilic leucocytes, on the other hand, remained below 100 cells for the first 4 months of life but reached their adult level, namely, 110 cells per c.mm., during the 5th and 6th months. Pearce and Casey (8) give a higher figure, namely, 214 eosinophilic leucocytes per c.mm.

The occurrence of certain accessory cells in the blood stream is indicated in Table I. The most interesting points are the presence of myelocytes in the circu-

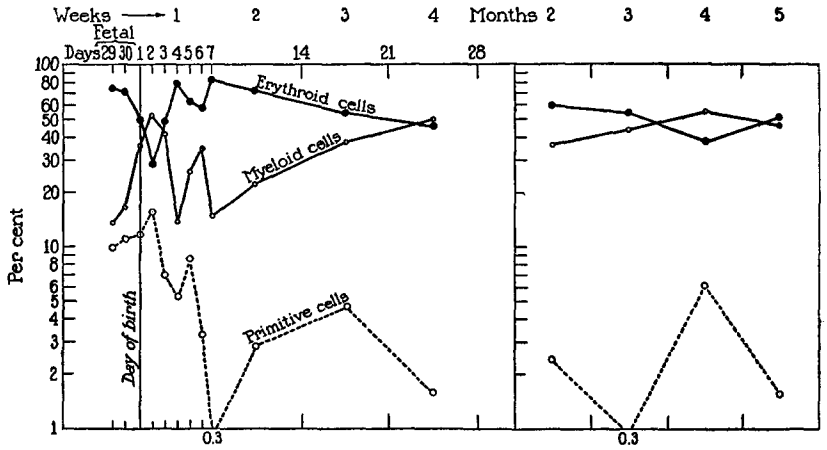


CHART 2. Three major strains of cells in the bone marrow of 49 young rabbits from data in Table II.

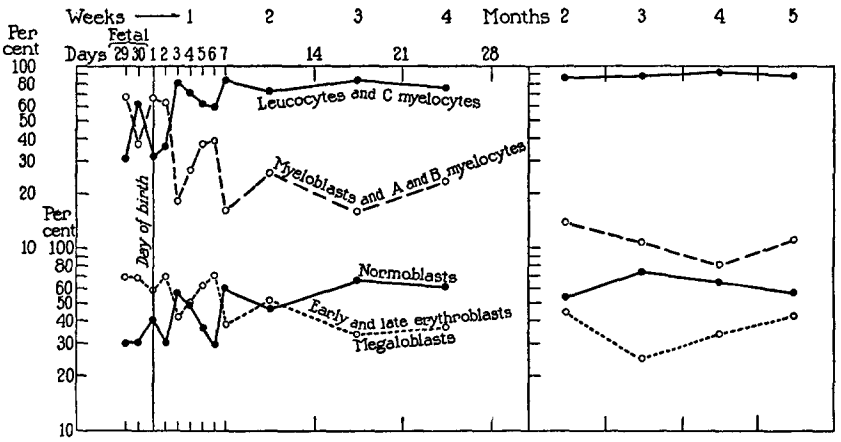


CHART 3. Relative proportion of mature to immature cells in both erythroid and myeloid strains of the bone marrow of 49 young rabbits from data in Table II.

lation in the fetal stages and on the day of birth. The blood of one of the animals, R 1292, counted on the day of birth, had a large number of megalokaryocytes, 8 per cent or a total of 168 per c.mm.

### *Differential Counts of the Cells of the Bone Marrow*

The records of the studies of the cells of the bone marrow are shown on Charts 2 and 3, from data given in Table II. Since there are no total counts of the cells of the marrow, the curves are plotted in percentages. The figures for Table II were obtained by taking the percentages of the total number of cells counted in all of the animals of a given age. Chart 2 gives the proportion of erythroid, myeloid, and primitive cells in the marrow, while in Chart 3 the data are analyzed to show the proportions of mature to immature cells in the erythroid and myeloid strains. For Chart 3 these two strains were considered as 100 per cent each and the percentage computed on that basis.

From the studies of the cells of the blood of the rabbit, it has been clear that the erythroid cells reach their final level much earlier than the myeloid types. This fact is reflected, or rather explained by the condition of the bone marrow, as shown in Chart 2. If the total numbers of cells in the marrow are considered, it is probable that there is a constant increase in both strains of cells but the disproportionate increase in erythroid cells during the 1st week of life is the most striking point in Chart 2. By the 4th to the 7th days of life the erythroid cells have reached an average of 70 per cent of the marrow cells. After the 7th day there was a gradual approach of these two strains of cells to approximate equality which was reached in the 4th week of life and maintained for the next 4 months (11, 12).

The counts of the cells of the marrow from the different bones of any animal have been, for the most part, consistent, and the irregularities in the curve of the early stages up to the 7th day have been due to single animals that have varied from the mean. The figures plotted for the 29th and 30th days, each from records of litter mates, showed the erythroid cells to be consistently high, varying from 57 to 82 per cent for the 29th day and from 52 to 86 per cent for the 30th. For one fetal rabbit, R 1170 A, we lacked a record of the date of mating but the litter was near maturity. The differential count is recorded in Table II but not plotted in Charts 2 and 3. The differential count showed a myeloid predominance.

On the day of birth, the counts of the erythroid cells in all 4 animals were close to the average, being 31, 40, 43, and 55 per cent respectively. The fall in the erythroid cells on the 2nd day was due to 2 animals, R 1779 and R 1780, in which the erythroid cells were 18 and 22 per cent respectively, while the other 4 animals had a range of from 42 to 69 per cent. On the 3rd and 4th days, all the counts were near the average; the drop on the 5th day was due to one animal only, R 4377, in which the percentage of erythroid cells was 21, while in the others the range was from 72 to 82 per cent. It is interesting to note that in an animal of the same litter as R 4377, counted the day before, that is on the 4th day of life, the average of erythroid cells was 75 per cent. Thus the drive toward erythroid cells was not initiated in every animal of a given litter at exactly the same age. The counts of the animals on the 6th and 7th days were consistent and near the mean. In general, of the 30 animals whose bone marrow was studied from late fetal stages through the 7th day of life, only 4 showed the erythroid cells below the myeloid.

The period of the 1st week of life is also significant in the preponderance of immature over mature cells in each of the two strains (13). This is shown on Chart 3. The erythroid cells are pictured in the lower half of the chart and it will be noted that, with the exception of the 3rd day, the immature cells were higher than the mature ones up to the 6th day of life. In the records for the 3rd day, the reverse was true for both of the animals counted, which were from the same litter. In the myeloid strain, on the other hand, the mature cells predominated earlier, namely, from the 3rd day onward.

### *Data from Study of Sections of Bone Marrow*

The study of the sections of the bone marrow in these stages adds important facts to those which can be obtained from the study of individual cells. In general, sections of bone marrow provide material from which to estimate the total amount of hematopoietic tissue. In late fetal life, the long bones are small and contain little marrow. The entire shaft contains spicules of bone with osteoclasts upon them.

On the day of birth, the bone marrow is relatively inactive (Fig. 1, from rabbit R 1627). The differential count in this animal was: erythroid cells, 65.8 per cent; myeloid, 18.5 per cent; primitive cells, 14.6 per cent, and accessory types, 1.1 per cent. Spicules of bone were present and the right hand one shows an osteoclast. By the 2nd day (Fig. 2) the spicules of bone had entirely disappeared, cleared away by osteoclasts. Such an osteoclast, shown in Fig. 3, gives significant evidence of its phagocytic function, being filled with particles which stained red in the eosin-methylene blue. On the 2nd day of life, the marrow (Fig. 2) had the sinusoids widely dilated and filled with blood corpuscles. Between the sinusoids the marrow cells were sparse. In this rabbit the differential count was: erythroid cells, 22.6 per cent; myeloid cells, 44.8 per cent; primitive cells, 31.2 per cent, and clasmatocytes and unclassified cells together, 1.3 per cent. The upper part of Fig. 3 is from the same preparation as the osteoclast shown in the lower part. This marrow also had about the same differential count as the one shown in Fig. 2. These 2 animals illustrate the fact that if the bone marrow is still relatively inactive, there may be a myeloid predominance.

Quite different is the proportion of the marrow cells to be seen 3 days later. The beginning of activity in the marrow due to erythroid hyperplasia is illustrated in Figs. 4 and 5 from a rabbit, R 1784, studied on the 5th day of life. Fig. 4 is a section of the tibia of this animal and Fig. 5 is from a film of this marrow stained in Wright's methylene blue-eosin. More than half of the marrow was hyperplastic, as is shown in the lower part of Fig. 4; the rest was like the marrow of the 2nd day, as is clear in the upper part of Fig. 4. The sinusoids were still filled with blood; there were no fat cells. The hyperplasia, which represents the fact that an enormous amount of cell division has taken place in 3 days' time, was due to the erythroid cells. The differential count was 73.2 per cent erythroid cells; 10.1 per cent myeloid; and 16.7 per cent primitive cells.

The further development of the erythroid hyperplasia and its liquidation are shown in Figs. 6 to 9′ from sections of bone marrow from the 2nd, 3rd, and 4th weeks of life. During the 2nd week the marrow becomes almost completely hyperplastic and fills the marrow cavity (Fig. 6). During this week erythroid cells were consistently high, the counts being 64, 67, 74, and 77 per cent respectively. This hyperplastic phase of the marrow is transitory, as shown in Figs. 7 to 9′. The rest of the 1st month is characterized by a rapid development of fat cells, which is correlated with a rapid increase in the size of the marrow cavity. These factors serve to reduce the hyperplasia by a marked spacing of the marrow cells. The beginning of the dispersion of the marrow cells is shown in Fig. 7 from rabbit R 1234, studied in the 3rd week of life. The differential count of the marrow cells showed a slight preponderance of myeloid types. The proportions were: erythroid, 34.3 per cent; myeloid, 55.9 per cent; primitive, 9 per cent; and clasmatocytes, 0.6 per cent. In the section the occurrence of the fat cells is clear.

Figs. 8, 8′, 9, and 9′ are all from the same animal, R 2048, from the 4th week of life. They show a reduction of the hyperplasia due to the growth of the bones and the consequent increase in the size of the marrow cavity. This stage of the development of the bone marrow represents a natural simplification of this organ which makes it possible to study to advantage the relationships of the cells of the primitive types to the myeloid and the erythroid strains. The connective tissue cells occur in two forms. Scattered throughout the bone marrow are cells of the size and type of the reticular cell or the fibroblast. Their nuclei are oval and have little chromatin; their cytoplasm is branched and shows little reaction to either basophilic or acidophilic stains. Since during the 4th week the fat cells are appearing in great numbers, it is easy to see that some of them become the fat cells. Others remain as fibroblasts. More important, from the standpoint of hematopoiesis, is the type we have recorded under the name primitive cell. This cell more nearly resembles the small lymphocyte than any other type. Several of them are indicated by arrows in Fig. 8′. One is marked by the left arrow in Fig. 9, in which it will be noted that the nucleus is poor in chromatin, showing only as a few tiny granules along the inner border of the nuclear membrane. Another primitive cell is shown as *C* in Fig. 9. In the living state this cell shows fewer signs of differentiation than the lymphocytes of lymph nodes, spleen, and blood. The cytoplasm of lymphocytes usually contains a few vacuoles which stain with the neutral red. Moreover, mitochondria, usually in the form of rods, easily seen without any stain and reacting readily to vital Janus green, are characteristic. In the primitive cells, on the other hand, as seen in this material from bone marrow, the narrow rim of cytoplasm contains almost no visible granules of any sort. The cytoplasm has little basophilia, and mitochondrial material must be finely divided. It is probable that in our differential counts of marrow cells we have underestimated the number of primitive cells on account of a tendency to select zones for enumeration in which the major strains of cells are clear and predominant. However, our records show (Chart 2) that up to the 5th day these cells are consistently above 5 per cent.

In supravital and fixed films of these early marrows we have found a few cells of this primitive type containing a few specific neutrophilic granules. They are clear in the sections of the marrow of rabbit R 2048. The primitive cell, to which the upper arrow of Fig. 8′ points, contains a few neutrophilic granules, as is clear at higher magnification in Fig. 8. These small granulocytes are fewer than the typical myeloblasts and myelocytes A, but they are important in connection with granulopoiesis, and will be considered in the discussion. The right hand arrow of Fig. 9 points to a myeloblast, which shows the increase in chromatin of the nucleus in this cell from the stage of the primitive cell.

This material from the 4th week of life is also significant for the study of the place of origin of both myeloid and erythroid strains. The extravascular origin of the granulocytes is well established, so the position of primitive cells, myeloblasts, and myelocytes outside the vessels, as shown in Figs. 8′ and 9′, but illustrates a well known point.

Concerning the red cells, on the other hand, there are marked differences of opinion. In Fig. 9′ is shown what we interpret as an erythrogenic capillary containing normoblasts. It lies obliquely across the upper part of the figure; in the upper left hand corner of this section is a mass of late erythroblasts or normoblasts, marked *A*, lying between two endothelial nuclei. Just to the right of them are two more endothelial nuclei, labeled *B*, the upper one of them being markedly swollen. Between these two nuclei is some material out of focus which is a clasmatocyte along the border of the vessel. Farther to the right, the endothelial wall of the vessel is still evident, with two leucocytes also out of focus, while beyond is an erythroblast, *B′*, at the beginning of a sinusoid, cut off in the photograph. The lower half of the section is occupied by myelocytes, mainly of Type B. One myeloblast in division is seen near the right hand border and near it is a primitive cell, marked *C*.

Further evidence on the placing of the red cells in bone marrow is shown in Figs. 10 to 13. The upper figures are from the bone marrow of rabbit R 1460, of the 2nd month of life, and the two lower figures from rabbit R 1306 from the 3rd month. Figs. 10 and 12 at the left, taken at low magnification, show that the marrow is not yet as packed with cells as it becomes in adult life. This is also especially marked in the border of the marrow, as shown in Fig. 11. The border later becomes densely packed with myelocytes, but at this stage they are almost lacking and thus the placing of the red cells is unmasked. To the left is a mass of erythroblasts and normoblasts, and the edge of the capillary in which they are contained is marked *A*. Other masses of normoblasts are marked *B*. In Fig. 13, a large sinusoid marked *A* passes obliquely across the field. The endothelial nuclei are clear as well as the contained erythrocytes. In the upper part of the figure is a large mass of erythroid cells, marked *B*, normoblasts and erythroblasts at what we interpret as the opening of an erythrogenic capillary, while on the lower right corner is a mass of normoblasts separated from the lumen of the sinusoid.

## DISCUSSION

The most striking point brought out in this study is the speed with which the young rabbit is able to produce the number of red blood cells characteristic of the blood of the adult animal in contrast to the delayed production of the corresponding number of white blood cells. By the 3rd week of life the rabbit's blood has its full quota of red blood cells, while the ultimate number of white blood cells is not reached until the 6th month.

Recent studies of red cells indicate two mechanisms, one for the elaboration of hemoglobin and the other for multiplication of red cells. In the embryo the mechanism for the elaboration of hemoglobin is more efficient than for the manufacture of the cells, but soon after birth the materials which stimulate the multiplication of red cells become available. The study of the bone marrow indicates that as this organ begins to assume function at the time of birth, the predominance of its activity is in the erythroid series, a sign of the imperative need of the body for hemoglobin. Thus the immediate activity of the marrow delivers to the blood stream rapidly increasing numbers of red cells but for the 1st month no increase whatever in granulocytes.

It has long been established that the first strain of blood cells to appear in both avian (14–16) and mammalian (17) embryos is the erythroid; and of mammalian embryos it is known that the white blood cells remain low in the blood stream throughout fetal life. This disproportion is shown in our data of the last few fetal days of the rabbit, where the white blood cells average only 900 per c. mm., or about 10 per cent of the number in the adult, while the 3,000,000 red cells of the same period are more than half their final number.

At the time of birth there is an increase in both strains of cells in the blood stream, probably due to a flooding of cells into the blood vessels from the sinusoids of the liver as its circulation is changed from the fetal type. In the case of the red cells, the increase to the circulation is of a million cells per cubic millimeter. The animals are, however, not able to maintain this increase in red cells during the 1st week of life, due perhaps in part to the sudden cessation of erythroid function in the liver and a lag period before the bone marrow can assume function. Another factor in the fall in red cells during the 1st

week of life may be an increase in blood volume, for which a stable number of red cells, or even a slight increase, cannot compensate.

The bone marrow, on the other hand, shows signs of a remarkable concentration of the factors which stimulate both the multiplication of red cells and the elaboration of hemoglobin. In late fetal stages the marrow is relatively quiescent and usually predominantly erythroid. Immature red cells are in greater numbers than mature ones, so the marrow cannot give many erythrocytes to the circulation. The time of the initiation of erythroid activity varies somewhat even in the same litter; it may start on the day of birth or be delayed until the 4th day, but is in full swing during the last half of the 1st week. This leads to a marked erythroid hyperplasia of the bone marrow during the 2nd week. The activity of the bone marrow from the 5th to the 7th days of life is not adequate to deliver enough mature red cells to the circulation to raise their number on account of the time needed for maturation; but during the 2nd week the marrow succeeds in increasing the red cells in the blood by half a million per cubic millimeter.

The appearance of hyperplasia in the bone marrow during the 2nd week of life means that for a short time the animal has to use much of the available space in the marrow cavity for the production of red cells. In the adult animal there are three mechanisms available to increase space for the formation of blood cells. The first is the shifting of fat from the bone marrow. The fat within the fat cells of the marrow is in a labile state; reversing the method of its formation, the fat readily breaks up into small droplets, leaves the cells, and passes into the blood stream. The second method is a thinning of the bone to make the cavity larger. During the 2nd week of life it is the growth of the bone and consequent enlarging of the marrow cavity that is the major factor in the adjustment. This means that the control of the growth of bone in young animals has a bearing on erythropoiesis. The cause of this adjustment must be chemical and perhaps of the nature of an endocrine balance; but one factor in the method for increasing the size of the marrow cavity is the formation of osteoclasts along its edge. It may be that a marked crowding of the marrow may favor the fusion of the osteoblasts into the giant cells that erode the bone. The mechanism for bone regression as well as for bone growth

is, of course, cellular. The rapid development of fat cells in the bone marrow during the 3rd and 4th weeks of life in the rabbit occurs during a period of rapid growth of the bone. The third method of increasing space for the formation of blood cells is the use of extramedullary zones.

From these observations it is clear that the first 3 weeks of life are crucial for the study of red cells in the rabbit. During this period the specific substances for the multiplication of red cells and the building up of hemoglobin must be present in high concentration. This mechanism is correlated with the factors that control the growth of bone. Thus the erythroid hyperplasia, which was built up so rapidly, is reduced almost as quickly, as can be seen by comparing Fig. 6 from the 2nd week with Fig. 8′ from the 4th.

One of the differences in opinion concerning the development of blood cells at the present time has to do with the place of origin of the red cells. It is in general agreed that in early embryonic stages in both birds and mammals the red cells arise within the vessels (1, 14–16). In mammalian forms, Maximow (17) judged that in the adult stages the evidence indicates that the red cells develop extravascularly and, when mature, break through the walls of the vessels to enter the blood stream. Recently, Jordan and Johnson (18) have expressed the same view for the adult pigeon. On the other hand, Doan (19, 20) and Doan, Cunningham, and Sabin (21) consider that the evidence indicates that in both avian and mammalian bone marrow red cells arise throughout life in collapsed capillaries. The opening of these erythrogenic capillaries into the sinusoids when erythrocytes are mature is the most feasible theory yet formulated for the entrance of these cells into the circulation. This is true since the erythrocyte does not possess the power of active locomotion. In the simplified marrow of the 4th week it is easy to find masses of developing red cells which appear to be surrounded by walls that may be interpreted as endothelial borders. The point cannot be made out for every group of developing red cells, especially when red cells are closely placed against myelocytes. This seems to us to be inevitable since in no other organ can the full capillary bed be determined without injection.

This study of the development of the white blood cells has brought out the fact that the leucopenia characteristic of fetal stages is overcome slowly by the rabbit and disappears only by the 6th month of

life. Moreover, our records show that each of the three strains of white cells, granulocytes, lymphocytes, and monocytes, increases in the blood stream at a different rate. For the 1st month of life the bone marrow is unable to initiate an increase in neutrophilic leucocytes. This is due to the fact that the size of the marrow cavity is only adequate to provide for the increase in the erythroid elements. By the 4th week, however, the marrow cavity has expanded so that the hyperplasia has been reduced and the space available in the marrow cavity gives a wide margin of safety. Though the marrow is now carrying on an amount of erythropoiesis proportionate to that which is characteristic of the adult, it is only just beginning to produce enough leucocytes to cause an increase in their number in the blood. At 4 weeks the number of neutrophilic leucocytes in the blood, 1,508 per c. mm., is not quite half their final number (20, 21).

The simplified state of the bone marrow during the latter part of the 1st month of life in the rabbit gives important material for the study of granulopoiesis. The second question at issue in hematology concerns the nature of the stem cell. It is accepted that there is a common stem cell for all the white blood cells. The only question at issue is whether this stem cell is identical with the lymphocyte or is a less differentiated type. It is clear that the stem cell is the lymphoidocyte of Pappenheim (22), or the lymphoid hemoblast of Jordan and Johnson (18). We have presented evidence for the theory that this cell, though it looks much like the small lymphocyte, lacks certain signs of differentiation. The differences though meager are worthy of consideration. In the primitive cell there is less chromatin in the nucleus; there may be nucleoli; there is less visible structure to the cytoplasm and no demonstrable basophilic material. Mitochondrial material must be finely divided rather than in the form of rods as in lymphocytes. Besides these points, the so called primitive cell occurs in bone marrow diffusely scattered and not in germ centers.

The primitive cell is able to elaborate the specific granulations without passing first into the phase of the myeloblast. The number of the tiny granulocytes arising directly from the primitive cells in the bone marrow without passing through the myeloblastic phase was small in our material (Figs. 8, 8′, 9, and 9′), as compared with the myeloblasts and early myelocytes. These small granulocytes may remain as abor-

tive myelocytes. In 1929, Smith and McDowell (23) described the occurrence of such small basophilic leucocytes in normal human blood and we have seen tiny granulocytes with neutrophilic granules in rabbits' blood. On the other hand, the primitive cell which has elaborated a few granulations may then enlarge and become a typical myelocyte.

It is quite clear that the occurrence of any granulations in these primitive types may be interpreted as evidence of the origin of granulocytes from lymphocytes (24–29) and we do not wish to stress a difference without a distinction, nor one which is merely to be resolved by terminology. The real distinction concerns the matter of potentialities of lymphocytes. In the one theory the so called primitive cell is undifferentiated and has all the potentialities for developing into any form of blood cell, while the lymphocyte, as seen in lymph nodes, spleen, and in the blood stream, is a mature cell with a distinct functional rôle. If the lymphocyte is a mature functioning cell, with as definite a cycle of maturation as the granulocytic strains (30, 31), it is in the interests of clarity to keep the term primitive cell for the less differentiated type. If, on the other hand, the mature lymphocyte can function for a time as a lymphocyte and then become either a granulocyte or a monocyte, then the lymphocyte must be considered both as a mature functioning cell and as a stem cell at the same time. The latter seems to us improbable.

As is shown on Chart 2, the primitive cell occurs in greater proportion around the time of birth than in the normal marrow of later stages. In still earlier fetal stages, the primitive cell is the predominating type, giving a marrow which Maximow (1) named primary lymphoid marrow. Correlated with these facts, certain observations on pathological bone marrows from work in progress by Dr. C. P. Rhoads and Dr. D. K. Miller at The Rockefeller Institute are of great interest. We are permitted by them to state them as follows: Under three conditions the primitive cell becomes a prominent feature of the bone marrow. First, in aplasia, this cell becomes conspicuous in the bone marrow, not because it has increased in numbers but because the more differentiated cells have disappeared; second, in agranulocytosis, and third, in certain cases of anemia with leucopenia, primitive cells become increased in numbers.

Our data on lymphocytes and monocytes show that their numbers increase rapidly in the blood stream during the 1st of month of life. Thus neither strain shows any depression from the predominance of erythropoiesis, such as is suffered by the white blood cells which develop in the marrow. The monocytes, coming as they do from the primitive cells diffusely scattered in the connective tissues, are not affected by the changes that take place in the marrow at birth and are the first cells of the blood stream to reach their full quota. This is accomplished in the 1st week of life so that they even precede the red blood cells in reaching maturity. Our studies of the blood cells indicate that the lymph nodes of the rabbit develop rapidly toward functional activity during the 1st month of life and then more slowly for the next 5 months.

## SUMMARY

1. The full number of erythroid cells in the blood stream of the rabbit is reached by the 3rd week of life.

2. During this period, there is a predominance of erythrogenesis in the bone marrow.

3. During the 2nd week of life the bone marrow is in a state of hyperplasia owing to the needs of the body for blood and the small space available for the marrow.

4. This hyperplasia is reduced as the growth of the bone permits the marrow to spread. The control of the growth of the bones has an important bearing on hematopoiesis.

5. During the first 3 weeks of life, the chemical factors for the multiplication of red cells as well as for the elaboration of hemoglobin become available.

6. The amount of hemoglobin does not increase as rapidly as the number of cells, so that the macrocytic anemia of the fetus becomes reduced. The proportion of hemoglobin per red cell characteristic of the adult rabbit is reached by the 3rd month.

7. Further evidence on the intravascular origin of red blood cells is given.

8. The development of all of the white blood cells, with the exception of the monocyte, goes on at a slower rate than that of the red cells.

9. The monocytes reach their full number in the blood stream in

the 1st week of life; granulocytes and lymphocytes by the 5th and 6th months.

10. Each of the three strains of white cells has a different rate of development.

11. The question as to whether the stem cell or primitive cell is identical with the lymphocyte is discussed.

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## EXPLANATION OF PLATES

### PLATE 7

FIG. 1. Section of the bone marrow of the tibia of rabbit R 1627, day of birth, stained with Giemsa. It shows two spicules of bone and marrow cells between. The differential count of the marrow of this animal was erythroid cells, 65.8 per cent; myeloid cells, 18.5 per cent; primitive cells, 14.6 per cent; accessory types, 1.1 per cent. The left arrow passes between some mature erythrocytes and points to a group of nucleated red cells. The right arrow points to an osteoclast. × 180.

FIG. 2. Section of the bone marrow of the humerus of rabbit R 1780, 2nd day of life, stained with hematoxylin and eosin. The differential count of the marrow of this animal was erythroid cells, 22.6 per cent; myeloid cells, 44.8 per cent; primitive cells, 31.2 per cent; and clasmatocytes and unclassified together, 1.3 per cent. × 240.

FIG. 3. Two parts of a fixed film of bone marrow of rabbit R 1779, litter mate of preceding animal, 2nd day of life, stained with Wright's eosin-methylene blue. The lower half of the figure shows an osteoclast filled with granules which stained red and were probably phagocytized bone. On the lower part of the osteoclast is a small cell out of focus. The upper part of the figure shows a marrow predominantly myeloid. Six normoblasts are plain and the rest are myeloid cells. The differential count was erythroid cells, 19.3 per cent; myeloid cells, 67.7 per cent; primitive cells. 12.7 per cent; and accessory types, 0.2 per cent. × 750.

FIG. 4. Section of the bone marrow of the tibia of rabbit R 1784, 5th day of life, stained with hematoxylin and eosin. The differential count was erythroid cells, 73.2 per cent; myeloid cells, 10.1 per cent; primitive cells, 16.7 per cent. × 240.

FIG. 5. Fixed film of bone marrow of the same rabbit as in Fig. 4, stained with Wright's eosin-methylene blue. All but three of the cells are erythroid. *A* is a clasmatocyte containing three normoblasts; *B* is a myelocyte B; *C* is a myeloblast. × 1,000.

### PLATE 8

FIG. 6. Section of the bone marrow of the femur of rabbit R 1466, 13th day or 2nd week, stained with hematoxylin and eosin. It shows a hyperplastic state. The differential count of this animal was erythroid, 79.5 per cent; myeloid, 19.5 per cent; primitive cells, 0.5 per cent; and accessory cells, 0.5 per cent. × 240.

FIG. 7. Section of the bone marrow of rabbit R 1234, 3rd week of life, stained with hematoxylin and eosin. The differential count of the marrow of this animal was erythroid cells, 34.3 per cent; myeloid cells, 55.9 per cent; primitive cells, 9

per cent; clasmatocytes, 0.6 per cent. The fat cells are plain in the clear spaces. × 240.

FIG. 8. Part of the same section as in Fig. 8′, at a magnification of 1,200 diameters, to show a primitive cell containing a few neutrophilic granules in the cytoplasm. It is marked with an arrow and is the same cell as the one marked with the upper arrow of Fig. 8′. The cell marked *a* is similarly marked in Fig. 8′ and may be an endothelial cell against a mass of normoblasts.

FIG. 8′. Section of the bone marrow of the tibia of rabbit R 2048, 4th week of life, stained in Giemsa. Section is 4μ thick. The differential count in this animal was erythroid cells, 23.4 per cent; myeloid cells, 74.9 per cent; primitive cells, 0.3 per cent; and accessory cells, 1 per cent. *A* marks a group of normoblasts; the arrows point to primitive cells. × 350.

FIG. 9. Part of the section shown in Figs. 8 and 8′ but from a different area. It shows a typical primitive cell, marked by an arrow. The nucleus has tiny granules of chromatin against the membrane. The right arrow points to a myeloblast. × 1,200.

FIG. 9. Section of the bone marrow of the humerus of the same rabbit as in Fig. 8, stained with hematoxylin and eosin. Section about 8μ thick. *A* is opposite a group of normoblasts between two endothelial nuclei; *B* opposite two endothelial nuclei of the same erythrogenic capillary, the upper nucleus is swollen. The dark cells within the capillary just to the right are leucocytes out of focus. *B′* is an erythroblast. *C* is opposite a primitive cell and to the right of it is a myeloblast in division. In the lower half of the figure are myelocytes. × 1,000.

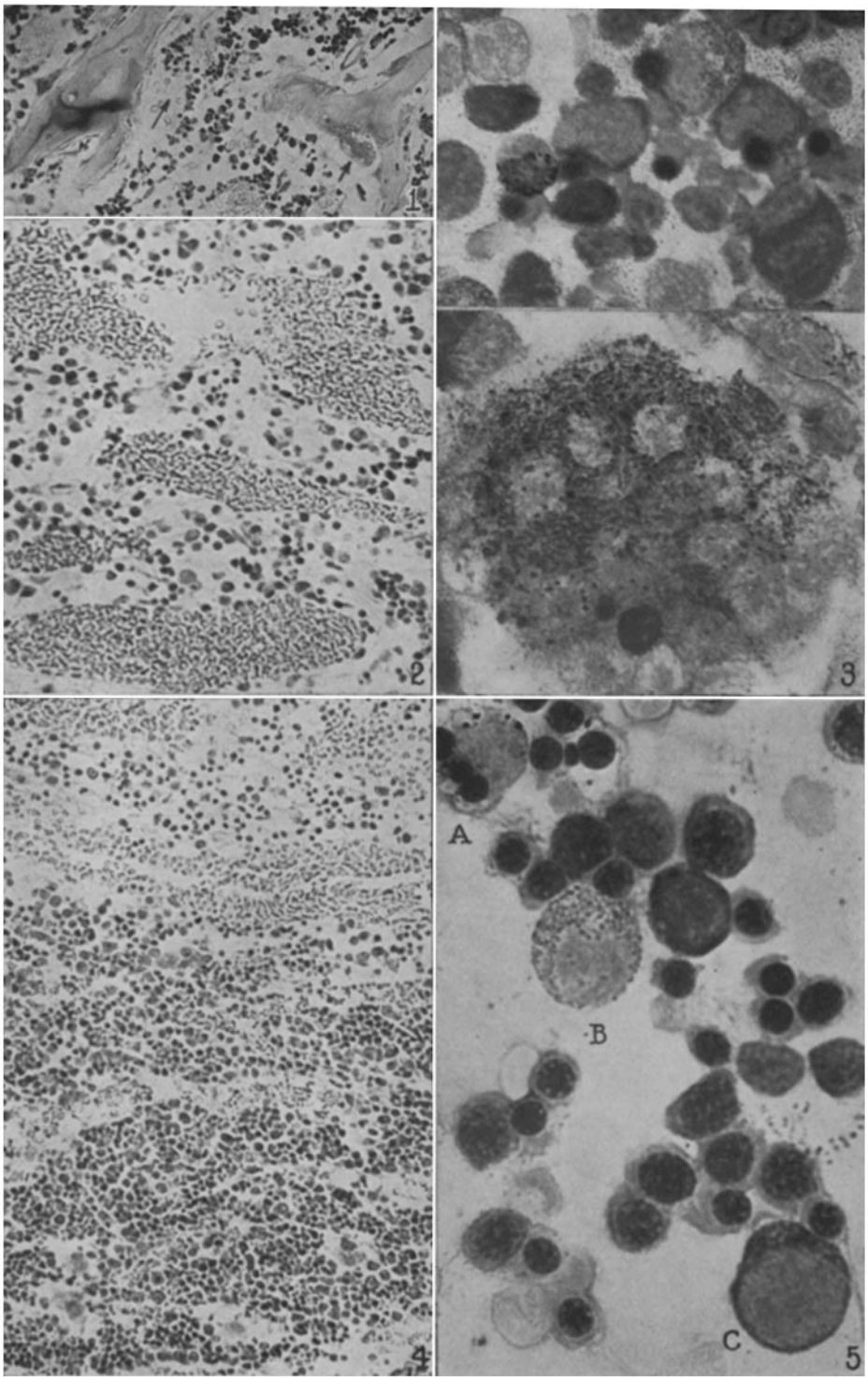
## PLATE 9

FIG. 10. Section of the bone marrow of the femur of rabbit R 1460, 2nd month of life, stained with hematoxylin and eosin. The differential count in this animal was erythroid cells, 73.4 per cent; myeloid cells, 24.2 per cent; primitive cells, 2 per cent. × 240.

FIG. 11. Section of the bone marrow of the femur of the same animal as in Fig. 10. *A*, endothelial wall around a group of normoblasts and erythroblasts; *B*, groups of normoblasts. × 1,000.

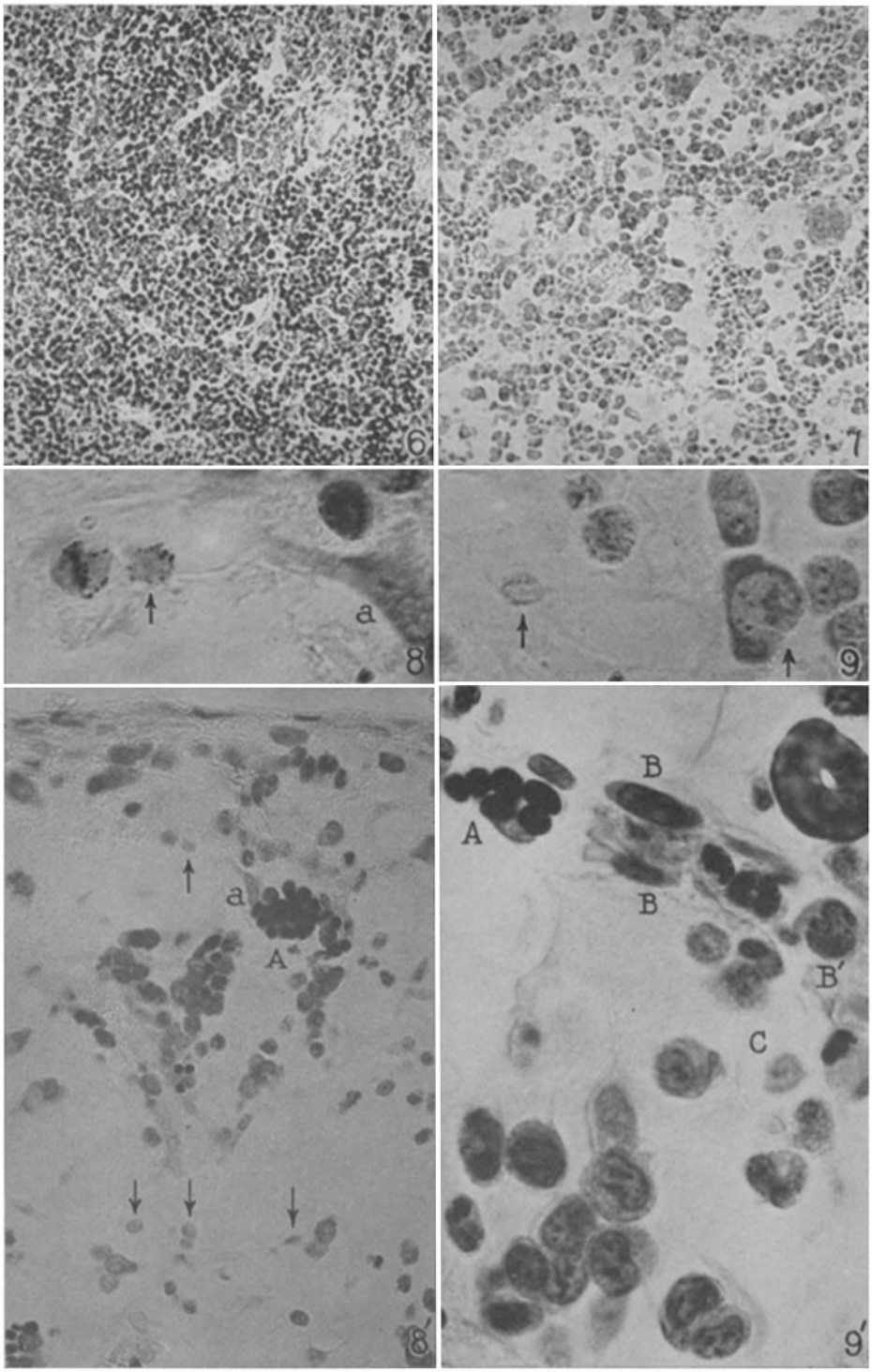
FIG. 12. Section of the bone marrow of the femur of rabbit R 1306, 3rd month of life, stained with hematoxylin and eosin. The differential count of this marrow was erythroid cells, 34.4 per cent; myeloid cells, 64.7 per cent; primitive cells, 0.6 per cent; and megalokaryocytes, 0.3 per cent. × 240.

FIG. 13. Section of the bone marrow of the femur of the same animal as in Fig. 12. Stained with hematoxylin and eosin. The letter *A* is in the lumen of a sinusoid; the upper letter *B* is opposite a mass of nucleated red cells in what we interpret as the opening of an erythrogenic capillary into the sinusoid. The lower letter *B* is a mass of nucleated red cells separated from the sinusoid by an endothelial wall. × 1,000.



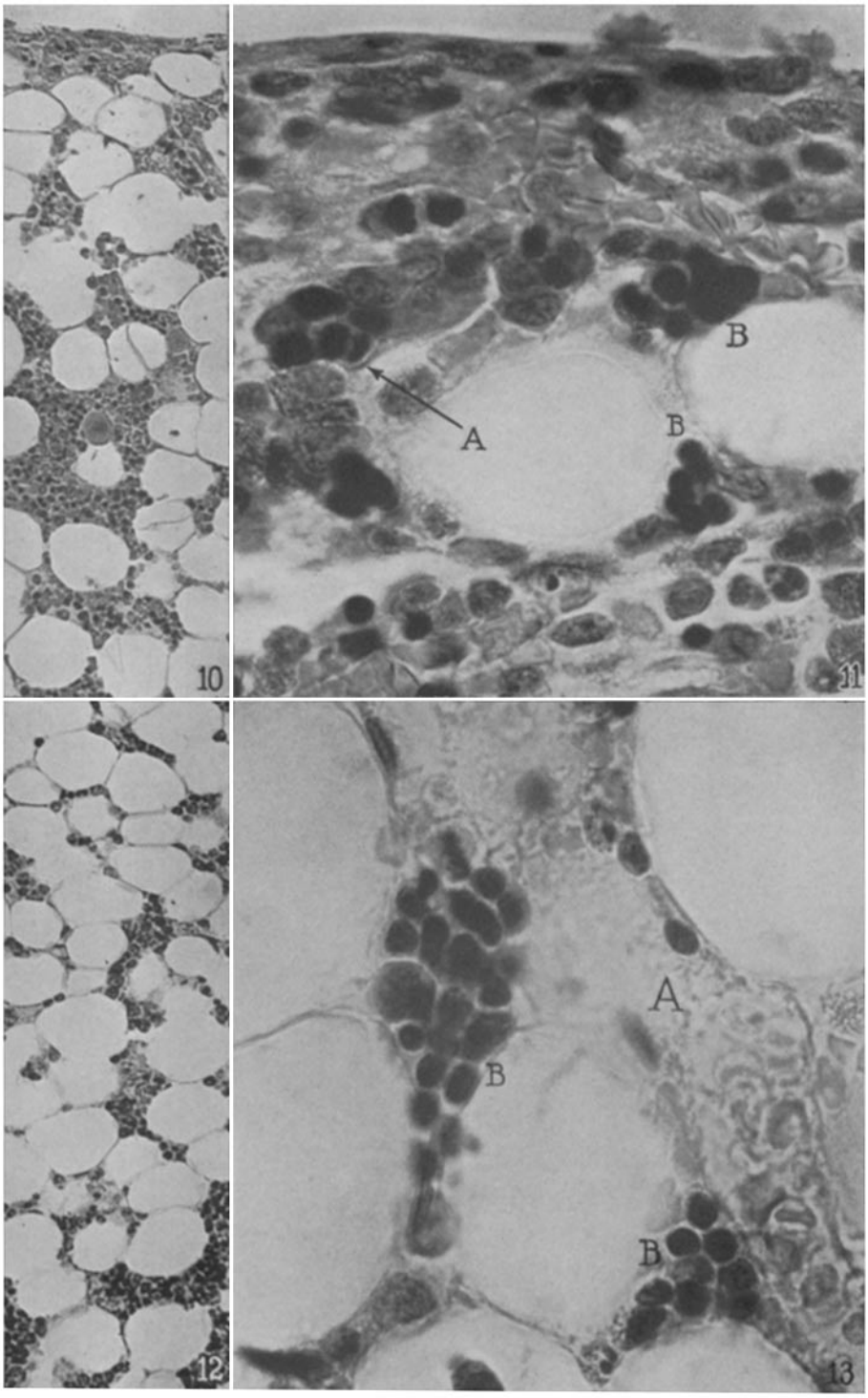

Photographed by Louis Schmidt

(Sabin *et al.*: Changes in bone marrow and blood cells)




Photographed by Louis Schmidt

(Sabin *et al.*: Changes in bone marrow and blood cells)

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(Sabin *et al.*: Changes in bone marrow and blood cells)