THE EFFECTS OF BLOOD LOSS AND BLOOD DESTRUCTION UPON THE ERYTHROID CELLS IN THE BONE MARROW OF RABBITS*

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The consideration of the bone marrow as a major organ of the body has recently been urged by Sabin and Doan (1–3). Although the component parts of this organ are widely scattered, and connected only indirectly through the blood stream, these parts tend to function as a unit. A continuous state of physiologic activity is maintained under normal conditions, reciprocal relationships existing between the various types of erythroid and myeloid cells of the circulating blood and their precursors in the marrow. The bone marrow also possesses a reserve power which is utilized in times of stress to produce more than the normal requirements of blood cells. Doan (3) pictures this reserve as existing in two distinct forms: first, the actual limited numbers of more mature cells ready for delivery into the blood stream, and second, the growth possibilities of the immature cells. He further states that a "shift to the left" or to more immature types of cells is the response to a demand for increased blood production.

Many investigators have been interested by the problem of erythroid marrow reserve, and numerous experiments have been done to determine the changes occurring in the marrow cells when the reserve power is activated. A productive organ such as the bone marrow can usually be studied most advantageously in relation to its product. Hence much of the experimental work on marrow reserve has involved the relationship existing between the pattern of the erythroid cells of the marrow and the peripheral blood picture.

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Price-Jones (4) rendered rabbits anemic by bleeding and by injecting phenylhydrazine, and examined smears of the bone marrow from animals killed in different stages and degrees of anemia. He found an increase in the number of nucleated cells over the normal more or less proportional to the degree of anemia. A marked erythroblastic reaction in the bone marrow, associated with the blood changes, has been noted by Muir and McNee (5) in rabbits made anemic by hemolytic serum. McMaster and Haessler (6) have reported that in rabbits repeatedly bled and reinjected with more than an equivalent amount of hemoglobin, the anemia was accompanied by an extensive spread of the red marrow with an increase in the size and abundance of the erythropoietic islands. Marrows of animals which were merely bled did not undergo these changes. Muller (7) has studied the effects of various colloidal substances on the blood and bone marrow. Small doses of collargol injected into rabbits produced erythrocytic marrow hyperplasia; larger doses finally produced an aplastic marrow with anemia. Injections of Weber's India ink gave rise to a persistent high degree of normoblastosis in the peripheral blood, associated with hyperplasia of the erythroid cell series in the marrow. Injections of gum shellac produced normoblastosis, erythroblastosis, and some degree of polycythemia in the circulating blood, with intense erythrocytic hyperplasia in the marrow. Robertson (8) found a decrease in reticulocytes in the marrow of rabbits rendered plethoric by transfusion, indicating a retardation of erythropoiesis. A severe but transient anemia occurred when the transfusions were stopped, the anemia being the result of a relatively inactive marrow. Sabin and Doan (1) have found that in normal rabbits the major supply of nucleated red cells is at the more mature stages (normoblasts 69 per cent, early and late erythroblasts 30 per cent, and megaloblasts 0.01 to 0.04 per cent); and Sabin (2) states that only in extreme anemia is the marrow thrown back to megaloblasts and endothelium.

Further experiments involving different methods of studying the relation between the erythroid cells of the marrow and the peripheral blood picture would be valuable. During recent years the number of young erythrocytes in the circulating blood, as determined by reticulocyte counts, has been recognized as a reliable index of erythrogenic activity in the bone marrow. By means of such reticulocyte counts it is possible to follow the level of red blood cell production in experimental animals with a reasonable degree of accuracy. The present investigation was designed to correlate the peripheral blood picture, especially the reticulocyte counts, with the pattern of the erythroid cells in the bone marrow. This study was made in normal animals and in animals in which blood production was increased by acute and chronic hemorrhage and by red blood cell destruction due to acetyl phenylhydrazine.

Methods

Healthy rabbits from standard stock were used in the experiments. Animals which showed evidences of infection or other abnormalities were discarded and the data were not included in the results. The animals were fed an adequate and highly nutritious diet and showed some gain in weight when observed for a period of several weeks.

At frequent intervals, usually daily or every other day, the peripheral blood obtained by puncture of an ear vein was examined. This included red blood cell count, hemoglobin determination, and reticulocyte count on a smear stained with brilliant cresyl blue and counterstained with Wright's stain. The absolute number of reticulocytes was calculated in each case and recorded in millions per cubic millimeter, giving a more accurate index of red blood cell production than the reticulocyte percentage. Smears were also examined for the presence of nucleated red blood cells and for changes in the size of red blood cells. Hemoglobin was estimated by the Newcomer method and was recorded in percentage. On the glass standard used, as checked by blood iron determinations, 100 per cent was equivalent to 15.6 gm. of hemoglobin per 100 cc. of whole blood. Occasional white blood cell counts were done to detect any gross abnormalities.

Blood volume was calculated on all animals subjected to hemorrhage, using the values given for rabbits by Went and Drinker (9). Hemorrhage was produced by incising one of the large veins at the base of the ear, the blood being received in a graduated cylinder to determine the exact amount lost by the animal. The incisions healed quickly, and the same vein could be used repeatedly for successive bleedings. Blood destruction was induced in some of the animals by the use of acetyl phenylhydrazine. The drug was used in a solution containing 15 mg. per cc., and was injected subcutaneously.

The animals were quickly killed by a sharp blow on the back of the neck. One femur was immediately removed and split open. A small cross-section of marrow 2 or 3 mm. long was taken from the upper end of the shaft of the femur. This piece of marrow was at once emulsified in 2 per cent sodium citrate and smears were made according to the method described by Doan and Zerfas (10). These cover-slip preparations were stained with brilliant cresyl blue at the time of smearing and were usually counterstained with Wright's stain. Some of the smears were counterstained with Jenner-Giemsa stain. Differential counts of 2,000 to 4,000 cells of the erythroid series were made on the smears of each marrow. The cells were divided into four groups: (*a*) mature erythrocytes, (*b*) reticulocytes, (*c*) normoblasts, and (*d*) erythroblasts and megaloblasts. The classification followed is that of Sabin (11).

The entire marrow of both femora and both tibiae was examined routinely. Longitudinal and cross-sections of femoral and, occasionally, of tibial marrow were fixed in Helly's fluid, sectioned, and stained with hematoxylin-eosin and Giemsa stains. Small portions of liver and spleen were also fixed, sectioned, and stained in the same manner.

Control Animals

The peripheral blood of six rabbits was examined frequently over a period of several days to establish normal blood values. Immediately after the final blood examination the animals were sacrificed and the bone marrow was studied. Condensed protocols are shown in Table I. The data on the peripheral blood of these animals compare favorably with the values given for normal rabbits by Scarborough (12). Although the average red blood cell count was high (5.14 million per c.mm.) there was a constantly maintained reticulocyte count which

TABLE I

Peripheral Blood Values and Percentages of Erythroid Cells in the Bone Marrow of Six Normal Rabbits

Rabbit No.	Peripheral blood values (average of all counts)			Peripheral blood values (immediately before death)			Erythroid cells in the bone marrow			
	R.B.C. per c.mm.	Hb	Absolute reticulocytes per c.mm.	R.B.C. per c.mm.	Hb	Absolute reticulocytes per c.mm.	Erythroblasts and megaloblasts	Normoblasts	Reticulocytes	Mature erythrocytes
	millions	*per cent*	*millions*	*millions*	*per cent*	*millions*	*per cent*	*per cent*	*per cent*	*per cent*
1	5.23	72	0.14	5.38	75	0.14	6.3	16.3	28.2	49.2
2	4.80	75	0.12	5.00	72	0.16	5.8	15.8	24.8	53.6
3	5.13	71	0.09	5.16	74	0.10	7.3	17.2	29.6	45.9
4	5.24	62	0.12	5.40	62	0.14	7.1	19.2	25.3	48.4
5	5.37	65	0.13	5.51	69	0.11	7.5	24.1	31.3	37.1
6	5.09	66	0.14	5.11	67	0.17	5.2	21.4	30.5	42.9
Average . .	5.14	69	0.12	5.26	70	0.14	6.5	19.0	28.3	46.2

averaged 0.12 million per c.mm. The red blood cells usually showed a mild but distinct degree of anisocytosis. Nucleated red blood cells were never seen in the peripheral blood smears.

The differential counts of the erythroid cells in the femoral bone marrow of these six animals were relatively uniform (Table I). The average of the six counts revealed that out of the total number of erythroid cells nearly one-half were mature and over one-fourth were reticulocytes, indicating that three-fourths of the erythroid cells were ready for immediate delivery into the blood stream. Only one-fourth of the cells were in the less mature nucleated stages of development.

Late erythroblasts comprised much the greatest proportion of the erythroblast-megaloblast group; megaloblasts were extremely scarce.

In the gross, the marrow of these animals was firm and red, with small flecks of yellow fat throughout the upper three-fourths of the femur and upper third of the tibia. The lower fourth of the femur and lower half of the tibia were filled with yellow fatty marrow. Some

TABLE II

Peripheral Blood Values, Bleeding Data, and Marrow Findings in Five Rabbits Subjected to Acute Hemorrhage

Rabbit No.	Average of peripheral blood values before bleeding			Bleeding data		Lowest peripheral blood values reached after bleeding			Reticulocyte peak (No. per c.mm.)	Peripheral blood values immediately before death			Erythroid cells in bone marrow			
	R.B.C. per c.mm.	Hb	Absolute No. reticulocytes per c.mm.	Total cc. of blood removed	Total per cent of blood volume removed	R.B.C. per c.mm.	Hb	Absolute No. reticulocytes per c.mm.		R.B.C. per c.mm.	Hb	Absolute No. reticulocytes per c.mm.	Erythroblasts and megaloblasts	Normoblasts	Reticulocytes	Mature erythrocytes
	millions	*per cent*	*millions*			*millions*	*per cent*	*millions*	*millions*	*millions*	*per cent*	*millions*	*per cent*	*per cent*	*per cent*	*per cent*
8	6.20	72	0.13	100	50	1.91	30	0.31	1.10	2.90	45	0.86	8.5	49.0	30.5	12.0
11	5.54	75	0.09	55	26	2.80	33	0.07	0.70	3.51	46	0.68	8.1	42.8	33.6	15.5
12	4.88	72	0.13	93	35	3.02	39	0.12	0.71	3.93	47	0.71	8.5	31.8	27.2	32.5
14	6.28	74	0.21	70	41	2.87	38	0.21	0.80	4.11	51	0.76	5.1	46.4	31.6	17.0
18	5.86	76	0.18	105	39	2.01	35	0.12	1.02	3.66	59	1.02	9.0	49.1	28.0	13.9
Average	5.75	74	0.15	85	38	2.52	35	0.21	0.87	3.62	50	0.81	7.8	43.8	30.2	18.2

slight individual variations in the extent of the red marrow were noted. Sections showed numerous large fat cells, even in the red marrow at the upper end of the femur, the marrow cells forming a network of thin strands and small clumps. Erythropoietic islands were seen but indistinctly.

The Findings in Acute Hemorrhage

After a period of several days for determination of normal blood values, the five animals included in this group were subjected to acute hemorrhage. In a single bleeding, or in two bleedings less than 12

hours apart, 26 to 50 per cent of the blood volume was removed. The animals were sacrificed at or near the peak of the reticulocyte response and the marrows examined. Condensed protocols are shown in Table II.

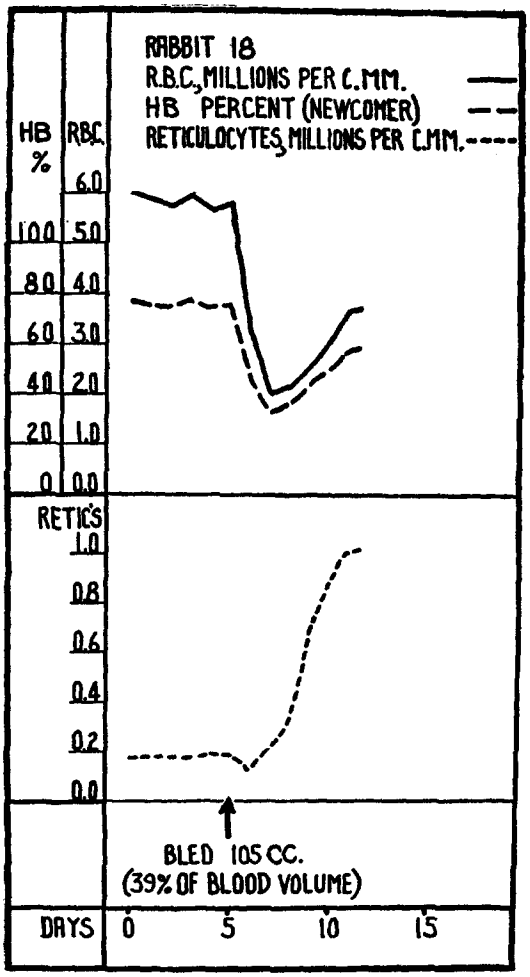

CHART 1. Observations of the blood in Rabbit 18 subjected to acute hemorrhage.

Chart 1 shows the typical course of blood values in an animal subjected to acute hemorrhage. Although an average of only 38 per cent

of the blood volume was removed by bleeding, the average lowering of the red blood cell count was 56 per cent. This discrepancy may have been due to overdilution or redistribution phenomena, or it may indicate that the total blood volume figures are too high. The reticulocyte response in the peripheral circulation, as determined by the increase in the percentage, began within a few hours after bleeding. Owing to the progressive lowering of the red blood cell count, the absolute number of reticulocytes per cubic millimeter did not show an increase until the 2nd or 3rd day after bleeding. Early in the response numerous abnormally large reticulocytes, heavily laden with hemoglobin, appeared in the circulating blood; later there were small as well as large reticulated cells. This gave rise to a high degree of anisocytosis. Nucleated red cells appeared sporadically when the reticulocyte counts were high, and they were never present in numbers large enough to include in a percentage count. The peak of the response, as closely as it could be determined, was reached on the 4th to the 6th day after bleeding and varied from 0.7 to 1.1 million reticulocytes per c.mm. At this peak the average number of reticulocytes per c.mm. was 0.87 million, or 5.8 times the prehemorrhage level of 0.15 million. At the time of death the level was slightly lower, being 5.4 times the normal.

The counts of erythroid cells in the femoral marrow of these animals (Table II) revealed a reversal of the normal proportions existing between mature cells, reticulocytes, and normoblasts. Normoblasts were the predominant type of cell, many of them large and heavily laden with hemoglobin. The percentage of erythroblasts and megaloblasts was not changed appreciably from the normal.

In the gross, the marrow of these animals was softer, redder, and more friable than that of normal animals. Little or no extension of red marrow into areas normally occupied by yellow marrow was noted, except in Rabbit 18 in which the red marrow filled almost the entire cavity of the femur. The small flecks of yellow fat seen in the red marrow of normal animals were less numerous or absent. Microscopically, sections of these marrows showed a perceptible decrease in the number and size of the fat cells. The erythropoietic islands were definitely enlarged.

The Findings in Chronic Hemorrhage

After a control period for determination of normal peripheral blood values, the four animals included in this series were subjected to a long course of bleedings. The animals were sacrificed 24 to 36 hours after the last bleeding and the marrows then examined. The condensed protocols are shown in Table III.

TABLE III

Peripheral Blood Values, Bleeding Data, and Marrow Findings in Rabbits Subjected to Chronic Hemorrhage

Rabbit No.	Average of peripheral blood values before bleeding			Bleeding data					Average of peripheral blood values during period of bleeding			Peripheral blood values immediately before death			Erythroid cells in bone marrow			
	R.B.C. per c.mm.	Hb	Absolute No. reticulocytes per c.mm.	No. of bleedings	Duration of bleeding period	Total cc. of blood removed	Total per cent of blood volume removed	Hb removed	R.B.C. per c.mm.	Hb	Absolute No. reticulocytes per c.mm.	R.B.C. per c.mm.	Hb	Absolute No. reticulocytes per c.mm.	Erythroblasts and megaloblasts	Normoblasts	Reticulocytes	Mature erythrocytes
	millions	*per cent*	*millions*		*days*			*gm.*	*millions*	*per cent*	*millions*	*millions*	*per cent*	*millions*	*per cent*	*per cent*	*per cent*	*per cent*
10	5.17	69	0.15	18	122	654	240	65	4.38	57	0.43	3.24	41	0.52	19.5	42.3	23.5	14.7
15	6.38	76	0.13	24	72	730	214	72	4.73	62	0.58	3.73	55	0.79	12.5	45.4	29.5	12.6
17	6.10	70	0.12	21	53	640	200	59	4.37	54	0.58	3.84	48	0.77	9.3	39.4	39.5	11.8
24	5.13	63	0.13	18	40	480	204	36	3.62	46	0.51	3.61	47	0.65	9.8	41.2	26.5	22.5
Average.	5.69	70	0.13	20	72	626	215	58	4.28	55	0.53	3.61	48	0.68	12.8	42.1	29.7	15.4

Measured amounts of blood were removed frequently in quantities sufficient to produce a sustained reticulocytosis without giving rise to a severe anemia. A total of 200 to 240 per cent (average 215 per cent) of the blood volume was removed; but, as suggested before, the blood volume values are possibly too high. Chart 2 shows the course of the peripheral blood values and the bleedings in Rabbit 15, which is typical of the series as a whole.

During the period of bleeding the average reticulocyte count was 0.53 million per c.mm., or approximately 4 times as high as the pre-

hemorrhage average of 0.13 million per c.mm. Immediately before death the average reticulocyte count was 0.68 million, or 4.8 times as high as the prehemorrhage level. At the termination of the period of bleeding, the average red blood cell count was 63 per cent and the average hemoglobin 69 per cent of their original values. Thus it seemed that, despite the average loss of 58 gm. of hemoglobin during

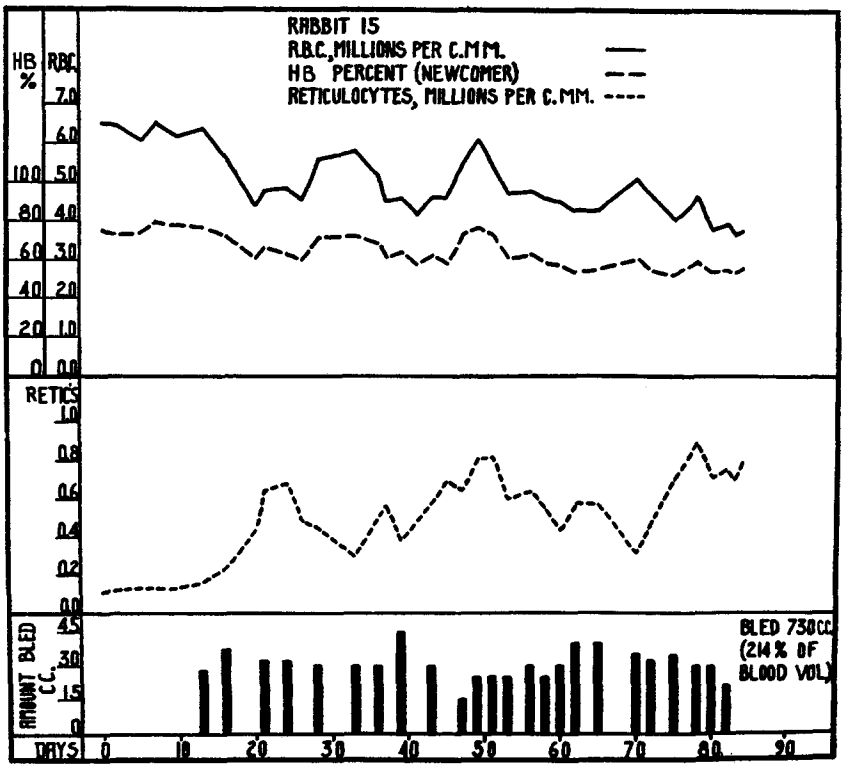

CHART 2. Observations on the blood of Rabbit 15 subjected to chronic hemorrhage.

the bleeding period, these animals were still able to produce red blood cells with a normal or slightly greater than normal quota of hemoglobin. Considerable anisocytosis was noted during the latter weeks. Nucleated red cells, always normoblasts, were frequently seen in the blood smears when the reticulocyte counts were high, yet they were never present in sufficient numbers to count.

Differential counts of erythroid cells in the marrow showed a reversal of the normal ratios between mature erythrocytes, reticulocytes, and normoblasts, being similar in this respect to the marrows of acute hemorrhage. Normoblasts were again the predominant type of cell. The percentage of reticulocytes was not appreciably changed from the normal; mature erythrocytes were much reduced. However, the most significant feature of the differential counts was the increase of erythroblasts and megaloblasts to nearly double the normal percentage. The rise in percentage of this group of cells was chiefly due to an increase in the numbers of late erythroblasts. Megaloblasts were present in small numbers.

The marrows of these animals were distinctly hyperplastic. The red marrow was soft and exceedingly friable, filling the entire shaft of the femur and the proximal half of the tibia. The distal half of the tibia contained fatty marrow, and a few small flecks of gross fat were seen in the distal fourth of the red femoral marrow. Sections showed the presence of fat cells in all but a few scattered areas of the red marrow, but these fat cells were greatly reduced in size and number. Erythropoietic islands were seen to be large and numerous, and, in many areas, confluent. The spleens of these animals were normal in the gross, but two of them showed some slight evidence of early extramedullary hematopoiesis.

The Findings in Phenylhydrazine Anemia

Following a period of several days, during which the normal blood values were determined, four animals were injected with acetyl phenylhydrazine to induce intracorporeal red blood cell destruction. Small doses of the drug were injected subcutaneously every 2nd or 3rd day in amounts sufficient to produce a sustained reticulocytosis. The animals were sacrificed and the marrows examined 24 hours after the last injection. Protocols are shown in condensed form in Table IV.

Chart 3 shows the course of the blood findings in Rabbit 19. This animal was given a relatively large initial dose of acetyl phenylhydrazine and responded with a high reticulocyte rise followed by an extensive drop in reticulocytes. Although this same phenomenon of abrupt rise and fall was noted in all four animals, those in which the

first dose was smaller did not show as high an initial reticulocyte rise nor as low a subsequent drop.

During the period of administration of acetyl phenylhydrazine the average reticulocyte count was 0.53 million per c.mm., a value 3.3 times as high as the average count in the control period. At the time of death the average reticulocyte count was 0.81 million, or 5 times the control average. At the end of the injection period, the average

TABLE IV

Peripheral Blood Values, Injection Data, and Marrow Findings in Four Rabbits Injected with Acetyl Phenylhydrazine

Rabbit No.	Average of peripheral blood values before injection			Acetyl phenylhydrazine injections			Average of peripheral blood values during period of injection			Peripheral blood values immediately before death			Erythroid cells in bone marrow			
	R.B.C. per c.mm.	Hb	Absolute No. reticulocytes per c.mm.	No. of injections	Duration of period of injections	Total amount injected	R.B.C. per c.mm.	Hb	Absolute No. reticulocytes per c.mm.	R.B.C. per c.mm.	Hb	Absolute No. reticulocytes per c.mm.	Erythroblasts and megaloblasts	Normoblasts	Reticulocytes	Mature erythrocytes
	millions	*per cent*	*millions*		*days*	*mg.*	*millions*	*per cent*	*millions*	*millions*	*per cent*	*millions*	*per cent*	*per cent*	*per cent*	*per cent*
19	5.78	63	0.25	16	41	163	4.24	60	0.59	4.45	68	0.94	17.1	32.3	28.8	21.8
20	5.29	56	0.16	16	41	152	4.09	52	0.53	3.86	51	0.63	17.6	33.7	29.6	19.1
21	5.14	72	0.11	18	40	201	4.26	63	0.47	3.18	53	0.80	20.6	51.9	21.6	5.9
23	5.48	64	0.13	18	40	172	4.15	55	0.53	3.51	53	0.86	17.6	46.5	26.5	9.4
Average	5.42	64	0.16	17	41	172	4.19	58	0.53	3.75	56	0.81	18.2	41.1	26.6	14.1

red blood cell count was only 69 per cent while the average hemoglobin was 87 per cent of the respective control period values. This relative increase of hemoglobin in relation to red blood cell count was paralleled by an increase in the average diameter of the red blood cells. There was also an increase in the hemoglobin content of many of the red cells, judging from their appearance in stained smears. The higher hemoglobin percentage might be accounted for by the fact that in the acute and chronic hemorrhage experiments there was a loss of iron, while, in the anemia produced by the administration of phenyl-

hydrazine, the red blood cells were destroyed within the body, thus retaining their content. In the latter weeks of the injection period

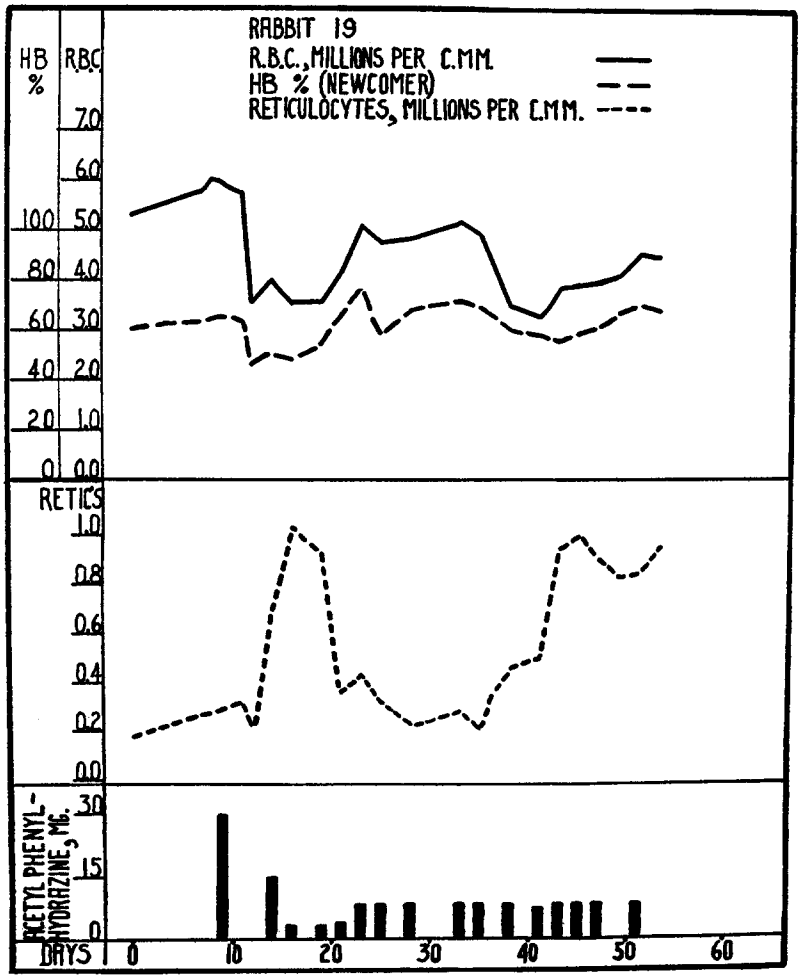

CHART 3. Observations on the blood of Rabbit 19 injected with acetyl phenylhydrazine.

the red blood cells showed a high degree of anisocytosis. Normoblasts were seen in a majority of the peripheral blood smears, but always in small numbers.

The differential counts of erythroid cells in the marrows of these animals again revealed the normoblast as the predominant type of cell. The percentage of reticulocytes was approximately normal; the mature erythrocytes were much reduced. The most striking fact shown by the differential counts was the great increase of the erythroblast-megaloblast group to 18.2 per cent, nearly 3 times the normal value for these cells. This increase was chiefly due to large numbers of erythroblasts, although megaloblasts were also frequently seen. Some disorganization of the erythropoietic process was suggested by the great variation in size of the erythroblasts and normoblasts.

In the gross, the red marrows of these animals appeared to be extremely hyperplastic, but did not show much more extension than did the marrows of chronic hemorrhage animals. Red marrow filled the entire cavity of the femur and the proximal half of the tibia, and in two of the animals small streaks of red marrow extended down through the fatty marrow in the distal half of the tibia. The marrow was rich red in color, somewhat darker than normal, and was exceedingly soft, being almost liquid in the upper third of the femur. No flecks of gross fat were visible in the femoral marrow.

In sections the fat cells were seen to be greatly diminished in size and number and in many areas they were entirely absent. Large confluent groups of erythrogenic cells were present in great numbers throughout the sections. Centers of intense myeloid activity were also evident.

The spleens of these animals were from 2 to 3 times the normal size and of a chocolate-brown color. The organs cut with much decreased resistance. An abnormally soft, mushy parenchyma bulged from the cut surface and no typical splenic structure could be seen. Sections of all four spleens showed distinct areas of erythropoietic activity. Although the livers were normal in the gross, microscopic sections showed a few small areas of toxic degeneration and leucocytic infiltration.

COMMENT

An interesting question of accuracy arises when an attempt is made to compare the high reticulocyte counts in the blood of animals having increased blood production with the reticulocyte counts in the same

animals before blood production was accelerated. Heath and Daland (13) have shown that reticulocytes probably mature to adult erythrocytes in from 4 to 6 days. In normal animals there is no urgent need for the liberation of immature cells into the circulation, and the maturation period of the reticulocytes is passed in part before leaving the marrow. Thus, in the marrow of normal animals, the reticulocytes tend to accumulate, and they greatly outnumber the normoblasts from which they are derived. On the other hand, animals that have suffered blood loss or blood destruction need every available red blood cell; reticulocytes are poured into the circulation soon after they are formed, and even an occasional normoblast escapes. In the marrow of these animals the reticulocytes, although present in a percentage approximating the normal, are much less numerous than their immediate precursors, the normoblasts. Therefore, when blood production is accelerated and the reticulocyte-normoblast ratio in the marrow is reversed, it seems probable that the reticulocytes pass the greater portion of their maturation period in the peripheral circulation. The high reticulocyte counts are, to some extent, due to this fact and are not entirely the result of increased cell production.

It must be borne in mind that certain errors are involved in the making of differential counts of the erythroid cells in the marrow. First, counts of cells in one standard portion of the marrow may not be representative of the state of the marrow as a whole. Second, in a series of cells which are rapidly maturing and constantly changing it is almost impossible to make wholly accurate classifications. This is especially true in the case of marrows in which erythropoiesis has been greatly accelerated and to a certain extent disorganized. Hence, differential counts of the erythroid cells in the bone marrow must be interpreted as indicative of general trends rather than as representative of absolutely accurate levels of red blood cell production.

The above experiments revealed four types of general trends in erythropoiesis. These types are graphically shown in Chart 4. The marrows of normal rabbits maintaining an average reticulocyte count of 0.12 million per c.mm. in the peripheral blood were characterized by the predominance of mature cells. The percentages of erythroid cells were, in fact, rather proportional to the degree of maturity of the cells. In all animals subjected to blood loss and blood destruction

there were definite changes in the marrow pattern. In animals studied during the recovery from acute hemorrhage, when the average reticulocyte count was at a peak 5.4 times the control level, the marrows showed a great diminution of mature erythrocytes, an increase of normoblasts to predominance, and no appreciable change in the percentage of erythroblasts and megaloblasts.

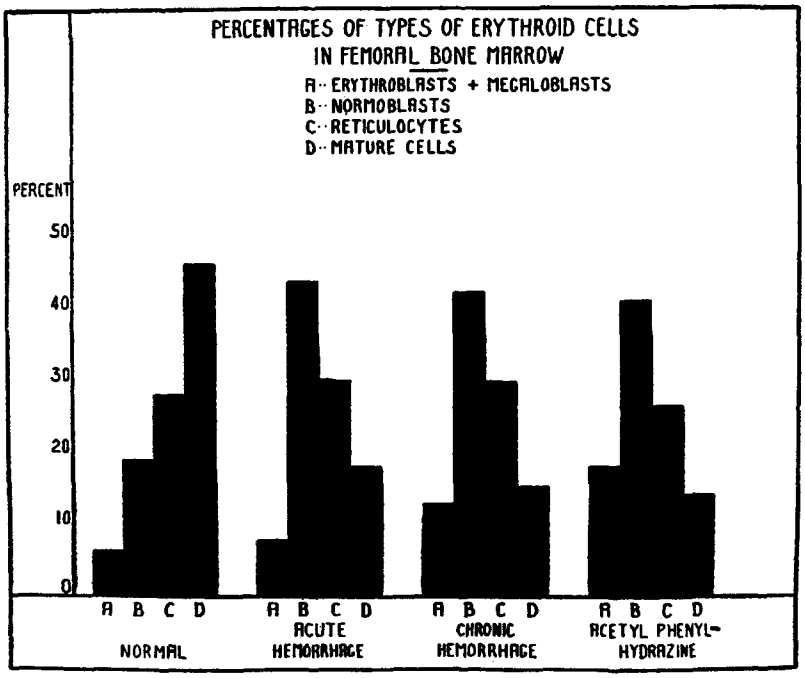

CHART 4. Percentage of types of erythroid cells observed in the femoral bone marrow of normal rabbits, of those subjected to acute and chronic hemorrhage, and of those in which acetyl phenylhydrazine was employed.

In animals which were subjected to chronic hemorrhage and which maintained an average reticulocyte count 4 times the normal for an average period of 72 days, the marrows were characterized by diminished percentage of mature erythrocytes, predominance of normoblasts, and double the normal percentage of erythroblasts and megaloblasts.

In animals which were injected with acetyl phenylhydrazine and

which maintained an average reticulocyte count 3.3 times the normal for a period of 40 days, the marrows were characterized by diminished percentage of mature erythrocytes, predominance of normoblasts, and nearly triple the normal percentage of erythroblasts and megaloblasts. The greater percentage of erythroblasts and megaloblasts may be the result of retaining the iron from the red blood cells destroyed within the body, in contrast to its loss in the chronic hemorrhage produced by the periodic removal of blood.

SUMMARY

The relative proportion of the various sorts of erythroid cells of the bone marrow has been determined after acute and chronic hemorrhage and after damage to the marrow with acetyl phenylhydrazine. The normal erythroid pattern shows megaloblasts and erythroblasts in the lowest percentage, then normoblasts, reticulocytes, and mature erythrocytes respectively, in increasing proportions. All three states studied show an increasing "shift to the left" up to a condition after acetyl phenylhydrazine, in which the erythroblasts and megaloblasts exceed the mature erythrocytes. The marrow pattern finds direct expression in terms of the cells of the blood.

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