THE FACTORS CONCERNED IN THE APPEARANCE OF NUCLEATED RED BLOOD CORPUSCLES IN THE PERIPHERAL BLOOD.

II. Influence of Procedures Designed to Increase the Rate of Blood Flow through the Blood-Forming Organs—Hemorrhage and Infusion.*

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In a previous paper the authors (1) have shown that the appearance of nucleated red corpuscles in the peripheral circulation is independent of the increased blood flow produced by exercise or by vasomotor paralysis. A slight increase in the number of these cells may be obtained, but one which, when thoroughly analyzed, never gives the picture of a true normoblast crisis. In the same paper it was said that the experiments on increased blood flow were designed to control more radical perfusion experiments by means of which cellular extrusion from the marrow could be studied directly. The interesting feature of normoblast appearance is its relation to blood regeneration, its reliability as an index of new formation. Perfusion work upon the marrow cannot be carried through a long enough period to give direct evidence of blood regeneration through increased red cell counts. It is therefore necessary to make use of the appearance of nucleated red cells as indicating the operation of stimuli for regeneration, provided this appearance can be shown to be an acute and reliable reaction.

In addition to a further demonstration of the fact that increased blood flow through the bone marrow has in itself no influence upon

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normoblast appearance, the present paper is designed to give a thorough picture of the nucleated red cell crisis, its time of appearance, duration, relation to marrow hyperplasia, etc. It would seem that nothing could be added to the literature of secondary anemia brought about by large, repeated hemorrhages. We had hoped on undertaking work upon the problem of blast appearance to find data which would place the reaction with complete accuracy. But such data do not exist so far as we have been able to ascertain. Tables giving the rate of regeneration in relation to hemoglobin formation are numerous, but neither these nor other studies give adequate pictures of the relation of nucleated red cells to regeneration.

Rieder (2) in his study of leukocytosis gives one instance in which normoblasts were found in the blood of a dog 24 hours after hemorrhage. This statement is unaccompanied by control figures of blood composition before blood loss.

Koeppe (3) finds nucleated red cells 23½ and 48 hours after hemorrhage in two rabbits. Here again there is lack of thorough examination before operation.

Zenoni (4) attempts to ascertain whether the appearance of nucleated red cells in the circulation is a result of the decrease of the blood mass through hemorrhage, or whether it is a hematopoietic reaction. Four dogs, two rabbits, and five guinea pigs were used, and care was taken that no normoblasts were present in the circulation before hemorrhage. The guinea pigs and rabbits were bled once from the carotid artery in large amount and films were made from the subcutaneous blood immediately and several hours after the operation. In one guinea pig normoblasts were found after 20 hours, in the two rabbits after 48 hours, and in one dog, treated in the same way, after 18 hours. No saline infusion was given in these cases. The remaining three dogs were bled repeatedly at one operation, the blood being defibrinated and returned. One showed normoblasts in 1½ hours, another in 5½, and the last in 7½ hours. Throughout the entire work there are no tables indicating the number of specimens examined, nor is there any note upon the character of the anesthesia. The hemorrhage and return of defibrinated blood involved a protracted operation. We have found that ether anesthesia, unless exceedingly brief, will cause the appearance of normoblasts with the familiar leukocytosis which is associated with this anesthetic. We may therefore summarize Zenoni's work in the conclusions that simple hemorrhage in rabbits, guinea pigs, and one dog required a considerable interval in order to cause blast appearance, and that rapidly repeated hemorrhages with return of defibrinated blood in dogs caused these cells to appear quickly. We have never repeated these last observations of Zenoni's, since they seemed to us to involve many possibilities aside from those he conjectured. The procedure undoubtedly gives repeated closing down and opening up of the marrow vessels as the circulation is depleted and replenished. This, of course, means periods of decreased and possibly increased blood flow, but unless done under sterile conditions, without defibrination and the consequent introduction of serum and hemoglobin with their possibilities of leukocytosis production and accompanying blast appearance, it seems to us to lack the controls necessary for conclusive deductions.

Lazarus (5) states that nucleated red cells do not appear in human blood until the 2nd or 3rd day after hemorrhage.

Von Willebrand (6) in a series of observations on posthemorrhagic anemia in dogs and rabbits makes the following comment:

Nucleated red corpuscles were observed after venesection in all the specimens of blood examined, but it must be stated in confirmation of the work of Timofejewsky, and Schauman and Rosenquist, that similar cells were found in three out of nine normal animals examined—dogs and rabbits—prior to venesection, although in these cases the nucleated cells were not numerous, the maximum being 4 in 10 sq. mm.

The cells were usually not present in large numbers after venesection. As a rule they were most numerous during the first few days following the operation, and then disappeared gradually from the blood. I have never been able to observe so numerous an invasion of these cells as von Noorden observed and described under the term "blood crisis."

The exact time of appearance of the nucleated red cells after bleeding cannot be readily determined, inasmuch as these cells may be present normally in the blood. In Experiment 3 relatively numerous cells (21 in place of 2 before venesection) appeared as early as $8\frac{1}{2}$ hours after the operation. All the nucleated cells were of the normoblast type. In size they resembled normal, unnucleated blood corpuscles.

Bunting (7) has compared the blood picture in a rabbit rendered anemic through repeated hemorrhages with that of ricin and saponin poisoning. He found 15 normoblasts per c. mm. 24 hours after the first of two hemorrhages totalling 20 cc. After 26 hours, 10 cc. more blood having been taken on the 24th hour, the number had risen to 108 normoblasts per c. mm. In a second case 12 cells were found 23 hours after a hemorrhage of 45 cc. In neither of these instances were counts recorded at short intervals immediately following the blood loss.

EXPERIMENTAL.

Dogs only were used in the experiments and since, as will be seen, the observations were carried over a long period, particular care was taken to keep the animals in good physical condition. They were fed on a diet of meat, bread, and dog biscuit. Except for an attack of acute and fatal distemper in Dog 1 none of the animals showed illness or suffered loss of weight. In addition to the five dogs whose records form the basis of this paper, we have bled with immediate infusion and bled without infusion six other animals, following the egress of cells at $\frac{1}{2}$ hour intervals for from 8 to 10 hours after the operation. As the results thus obtained coincide with those obtained with the five animals subjected to more or less continuous observation we do not include them specifically in any of the data presented.

Charts of three of the five dogs of this series are given in this paper (Text-figs. 1, 2, and 3). In the case of the first two, we did not at first realize the need for daily observations so that their records are incomplete, but as far as they go they amply confirm the published data.

It was our original intention to bleed and infuse, studying the blood composition before the operation and at repeated, short intervals after it Experiments with the isolated tibia of the dog showed that hemorrhage and immediate infusion, with consequent blood dilution, will increase the blood flow through the bone often fivefold. Could it be shown that the sudden increase dislocated marrow cells? We have already commented upon the fact (1) that if the marrow vessels of all mammals are as incomplete as those pictured for the rabbit, allowing the normal blood current to wander through a loose network of cells, there should be easy dislocation of such cells under the influence of a markedly increased blood flow. That the dislocation does not occur readily has been indicated by the literature cited, but not proved, because of lack of precise following of the blood changes. It is noticeable that in none of the experiments cited has there been hemorrhage with immediate saline infusion. It is a question how long blood volume restoration by intravenous salt solution lasts. But there is no doubt that for the 1st hour following hemorrhage and infusion the volume of blood passing through the bone marrow is greatly increased. Boycott and Douglas (8) have shown extremely rapid restoration of blood volume in rabbits subjected to large hemorrhages and not infused. Dogs apparently restore blood volume more slowly, though Hünerfauth (9) found no difference between dogs and rabbits. It is possible that this rapidity of restoration indicates the reason our experiments upon blast extrusion show no variation whether or not salt solution was given. There is a frequent small increase in nucleated red cells immediately after hemorrhage, determined apparently by the fact that all intravascular blood cells are in the active blood current at this time and the true blood composition can, therefore, be properly appreciated on examination of specimens from the capillaries.

Boycott and Douglas (8) emphasize the increase in speed of regeneration of hemoglobin which follows repeated hemorrhage and consequent extension of erythrocyte-forming tissue. The effect of hemorrhage in drawing immature red cells into the circulation has not been studied in animals which, because of marked hyperplasia, are known to have a large volume of cell-bearing tissue. The fact that normoblasts accompanying leukocytosis appear with peculiar readiness in children has been commented upon, but the literature is lacking in precise following of the reaction through a series of hemorrhages.

Technique and Immediate Effects of Hemorrhage and Infusion.

Unless otherwise designated blood specimens were taken by subcutaneous puncture after a brief period of exercise. In every case red cell counts, white cell counts, and two films were made, the latter being invariably stained with Wright's stain. Nucleated red corpuscles were enumerated per c.mm. on the basis of the number seen while counting 1,000 leukocytes in the films. All red and white cell counts were made by two of us.

It was found early in the work that the individual hemorrhages were better borne and could be made much more extensive if accompanied by sterile saline infusions. Consequently figures given for the amount of blood removed do not represent whole blood, since in the latter part of the hemorrhage much of the fluid taken out had just been introduced. Under ordinary circumstances after removal of from 200 to 400 cc. of blood an equal amount of salt solution was returned through the same cannula. Then followed another large blood removal and saline injection, and so on until in our judgment the danger limit had been reached. While this method proved most satisfactory for the purpose of protracted experiments when it was essential to run no risk of losing the animal, it obviously leaves the estimate of the size of hemorrhage to the blood counts. In nearly all cases morphine and cocaine anesthesia was used, ether being necessary but a few times.

All operations were done under sterile conditions. Two bleedings could usually be obtained from each external jugular vein, one from each of the carotid arteries, one from each brachial artery, and in large dogs one from each saphenous artery. This progressive cutting down of the vascular bed never produced permanent ill effects and gave opportunity for ten hemorrhages, more than enough for our purpose. On several occasions we saw temporary circus movements and amblyopia after large hemorrhages, but prompt recovery followed.

Tables I and II illustrate the immediate effect of hemorrhage and infusion upon the nucleated red cell count in a normal dog (Table I), and in the same animal rendered hyperplastic by four hemorrhages occurring at intervals during a period of $2\frac{1}{2}$ months (Table II). On

TABLE I.

Immediate Effect of Hemorrhage and Infusion upon the Nucleated Red Cell Count in a Normal Dog.

Dog 2; female; weight 8.9 kilos; October 21, 1916.

Specimen.	Hour.	Red cells per c.mm.	White cells per c.mm.	Nucleated red cells per c.mm.	Remarks.
	a. m.—p. m.				
1	9.35	4,960,000	12,700	127	1st experimental day.
	9.48				Ether begun.
2	10.09	5,488,000	18,500	203	Specimen 2 taken just before bleeding and after 21 minutes of ether.
Average......		5,224,000		165	
	10.09–10.23				360 cc. of blood removed in one large hemorrhage; 660 cc. of salt solution returned.
3	10.23	2,776,000	10,300	144	Taken immediately after saline infusion.
4	10.45	3,200,000	8,000	288	Animal rapidly coming out of ether.
5	11.30	3,504,000	11,900	154	
6	12.15	3,816,000	18,200	273	
7	1.00	3,400,000	19,700	157	
8	1.45	3,392,000	20,700	227	
9	2.30	2,992,000	20,200	141	
10	3.15	3,192,000	20,800	166	
11	4.00	3,000,000	17,800	142	
Average......		3,252,000		188	

October 21, 1916, Dog 2 in two observations showed an average of 165 nucleated red cells per c.mm. After a hemorrhage and an infusion of salt solution which reduced the red cell count from 5,224,000 to 3,252,000, nine observations during a period of 5½ hours immediately following the hemorrhage gave an average of 188 nucleated red cells per c.mm. of blood. This increase, which becomes relatively larger when considered from the point of view of proportion of nucleated red cells to total red cell count, is nevertheless too small, in our opinion, to be regarded as representing a real dislocation of cells from the marrow. We believe it to have been due to a profound stirring up of the circulation following the infusion, and to a consequent more even distribution of cells throughout the peripheral area—a phenomenon such as we have shown to occur with exercise (1). This belief is further strengthened by the figures given in Table II, taken from the same animal.

On January 15, 1917, Dog 2, rendered hyperplastic by four fairly severe hemorrhages, was again bled and infused. Two observations before hemorrhage showed no nucleated red cells. Twelve observations following the infusion and extending over a period of 6 hours gave an average of 5 nucleated red cells per c.mm. Ten of these twelve observations showed no blasts at all. This insignificant increase in an animal whose marrow was presumably richly provided with nucleated red cells, strengthened our belief that the increased rate of blood flow following hemorrhage and infusion is not a procedure which will dislocate cells from marrow.

Table III summarizes briefly the immediate results of numerous hemorrhages in Dogs 3, 4, and 1, giving the number of specimens taken before and after the hemorrhage on each experimental day, the number of hours after hemorrhage during which the observations were made, and the average red cell, white cell, and nucleated red cell count before and after each hemorrhage. These figures confirm those taken from Dog 2. In thirteen out of nineteen cases a slight increase occurred in the number of nucleated red cells following the hemorrhage and infusion; in five cases a slight decrease in the number of blasts followed the hemorrhage; in one instance there was no change.

TABLE II.

Nucleated Red Cell Count in a Dog Rendered Hyperplastic by Four Hemorrhages at Intervals during a Period of 2½ Months.

Dog 2 (continued); January 15, 1917.

Specimen.	Hour.	Red cells per c.mm.	White cells per c.mm.	Nucleated red cells per c.mm.	Remarks.
	a. m.—p. m.				
					Animal bled and infused on Oct. 21, 1916, Nov. 10, Dec. 14, and Jan. 1, 1917.
	8.50				1.2 cc. of 3 per cent solution of morphine sulfate subcutaneously.
1	8.55	7,528,000	11,500	0	
2	10.20	7,096,000	15,800	0	
Average......		7,312,000		0	
	10.20–10.40				Bled 630 cc. from external jugular vein; 1,100 cc. of salt solution infused.
3	10.40	2,432,000	17,200	0	
4	11.00	3,120,000	13,000	0	
5	11.30	3,284,000	17,600	0	
6	12.00	2,912,000	19,100	19	
7	12.30	3,176,000	19,400	0	
8	1.00	3,216,000	26,800	0	
9	1.30	3,286,000	24,250	48	
10	2.30	3,028,000	27,600	0	
11	3.00	3,008,000	30,100	0	
12	3.45	3,056,000	25,800	0	
13	4.30	2,944,000	28,000	0	
14	4.45	3,072,000	30,800	0	
Average......		3,044,000		5	

The True and the Pseudocrisis and the Time of Their Occurrence.

We have just pictured the immediate result of hemorrhage and infusion. On inspection of Text-figs. 1, 2, and 3 it is evident that the slight increase in nucleated red cells occurring immediately after

TABLE III.

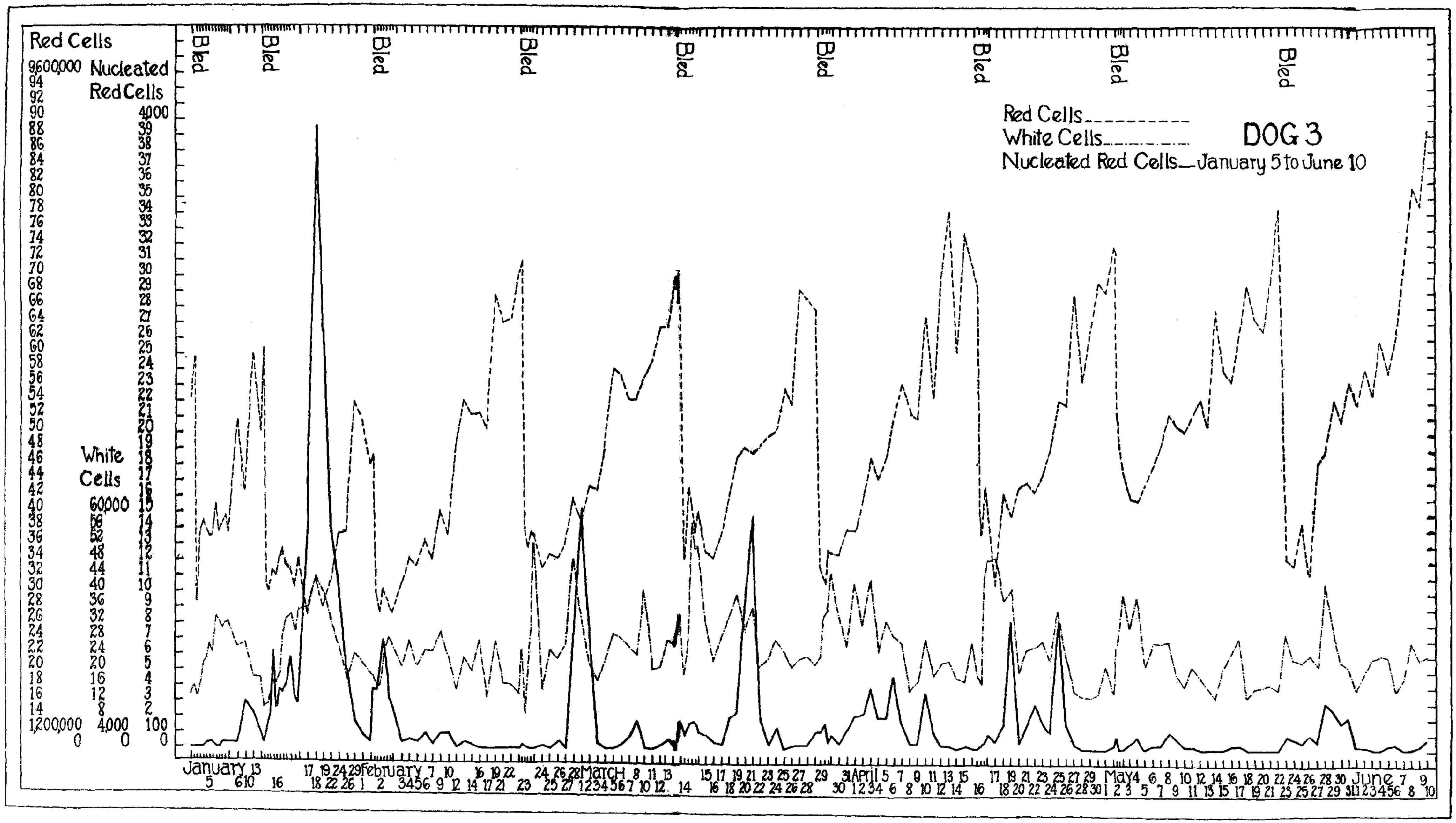
Immediate Effect of Numerous Hemorrhages upon the Red Cell, White Cell, and Nucleated Red Cell Count.

Date.	No. of specimens taken.		Duration of observation after hemorrhage.	Average No. of red cells per c.mm.		Average No. of white cells per c.mm.		Average No. of nucleated red cells per c.mm.	
	Before hemorrhage.	After hemorrhage.		Before hemorrhage.	After hemorrhage.	Before hemorrhage.	After hemorrhage.	Before hemorrhage.	After hemorrhage.
				Dog 3.					
1917			*hrs.*						
Jan. 5	2	11	5¼	5,696,000	3,653,000	14,700	25,000	0	15
" 16	2	10	6	5,556,000	3,261,000	14,000	23,700	59	347
Feb. 2	2	6	5½	4,664,000	2,852,000	18,400	22,300	206	450
" 23	2	4	3½	7,122,000	3,720,000	19,800	29,600	12	2
Mar. 14	2	5	6¼	6,788,000	3,928,000	30,300	42,400	85	133
" 29	1	4	5¼	6,592,000	3,378,000	21,400	36,300	107	102
Apr. 16	2	3	5	7,040,000	4,058,000	23,200	36,300	0	57
May 2	1	3	4	7,416,000	4,834,000	14,400	32,700	14	26
				Dog 4.					
Feb. 9	2	6	5¼	8,812,000	6,384,000	17,000	21,200	0	0
" 20	2	6	5	6,113,000	5,214,000	20,700	38,200	0	266
Mar. 9	1	4	4¼	5,928,000	2,618,000	34,100	28,100	0	47
" 28	2	4	5	6,120,000	3,001,000	14,300	18,300	0	25
Apr. 17	2	3	5	6,772,000	3,320,000	15,100	30,500	14	55
May 4	1	2	2¼	7,040,000	5,463,000	17,800	28,300	0	52
				Dog 1.					
Mar. 7	1	6	5	6,072,000	4,042,000	28,300	18,000	28	2
" 16	1	5	5¼	5,048,000	2,724,000	18,000	19,400	54	65
Apr. 2	2	4	4¾	5,860,000	3,688,000	21,300	28,700	205	89
" 18	2	2	2½	6,158,000	4,080,000	13,600	16,700	423	245
May 7	1	1	½	6,124,000	3,360,000	16,600	8,900	33	320

hemorrhage never reaches a height comparable with that attained later in the curves, just before pronounced red cell increase. This first small addition of normoblasts is well seen in Text-fig. 1, Dog. 3, February 2, 1917. The result of hemorrhage and infusion on this day has been to bring into the active circulation every available cell. The situation is comparable with that seen on immediate exposure to low barometric pressure when polycythemia occurs at once and expresses the full cell count of the animal at the moment. Later there is a formative polycythemia with the reestablishment of a reserve of red cells. In the same way, with the speeding up of the circulation which occurs immediately following hemorrhage and infusion, every available cell begins to circulate actively. The situation is exactly the one presented by exercise, and we designate the minor increases in nucleated red cells occurring after exercise and immediately after hemorrhage and infusion as pseudocrises. These pseudocrises do not foretell rapid regeneration and it is even doubtful in our minds whether the cells found in them really leave the marrow pulp. They are more probably in the circulation when the hemorrhage occurs.

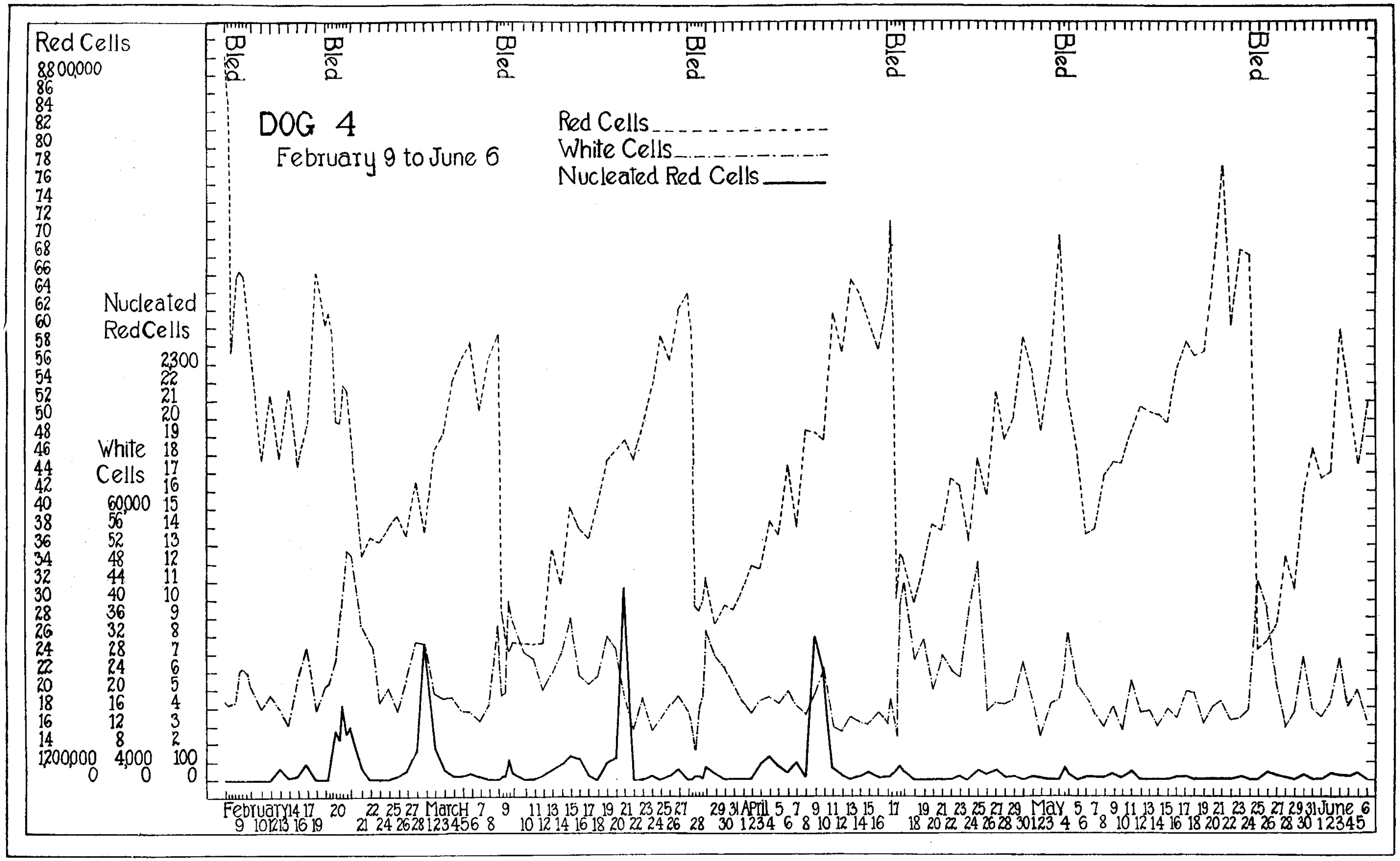
Other examples of the pseudocrisis are seen in Text-fig. 1, Dog 3, March 14 and 29. In these two instances the increases are less than on February 2 and they are preceded by periods of low normoblast content, while that of the earlier date is the result of hemorrhage just at the termination of an extremely large, true formative crisis. Even in the presence of the most advanced hyperplasia, evidenced by autopsy, the immediate effect of blood dilution and increased blood flow through the blood-forming organs is not to dislocate normoblasts but only to give a truer picture of the vascular content of these cells at the time of the operation.

True crises are easily recognized on the charts and their relation to red cell regeneration is obvious. They occur and disappear with great rapidity. This is particularly conspicuous in Text-fig. 3, Dog. 1. The first true crisis here begins on March 21 and ends on the 23rd; the second crisis, beginning April 13, lasts 3 days, in the first two of which the normoblasts increase from 434 to 8,210 per c.mm. It is plain that the crisis is always related to a favorable event, namely active red cell regeneration, but it is equally plain that the true formative crisis is not a necessary accompaniment of regeneration. Indeed, the transiency of the phenomenon, coupled with the fact that as animals are bled repeatedly they lose the tendency to show crises, emphasizes most strongly the fact that every effort is made to avoid this loss of nucleated red cells into the general circulation. A crisis usually occurs after the second hemorrhage. In the three charts presented this is marked. In two cases, Text-figs. 2 and 3, the crisis is largest after the third hemorrhage and then, with regeneration



TEXT-FIG. 1. Graphic picture of the daily red cell, white cell, and nucleated red cell counts of Dog 3 during a period of 5 months, showing the immediate and subsequent effects of nine hemorrhages. The ordinates represent cells per c.mm. of blood; the abscissæ represent days of the month. When a number of observations were made on the same day immediately following a hemorrhage and infusion, they are indicated by short vertical upstrokes on the line of the abscissæ included between two long upstrokes, which indicate the first and the last observation of the day. Single daily observations are indicated by single long upstrokes on the line of the abscissæ.

Contrast the pseudocrises of blasts immediately following hemorrhage and infusion with the true blast crises occurring during active red cell regeneration. Note the gradual development of the ability to regenerate without crises and the steadily increasing overregeneration of red cells as the hemorrhages continue.



TEXT-FIG. 2. Curve of the daily red cell, white cell, and nucleated red cell counts of Dog 4 during a period of 4 months, showing the immediate and subsequent effects of seven hemorrhages. The same features in the curve are to be noted as in Text-fig. 1. The initial high red cell count is probably due to a fright polycythemia.

occurring rhythmically and rapidly after succeeding hemorrhages, there is a progressive decrease in the size of the crises. This gradual acquirement of a power to hold nucleated red cells in the face of rhythmic blood loss is an adaptation which might be expected. While dogs frequently show many nucleated red cells in the peripheral blood and in this case usually display the largest crisis after the first hemorrhage, they invariably begin to regenerate without crises as bleedings are continued. It is unfortunate that in our experiments the hemoglobin content was not followed in relation to this phenomenon, since the failure of hemoglobin regeneration at a rate comparable with cellular regeneration must produce a considerable color anemia in animals treated as these have been. The presence of a low hemoglobin has, however, had no effect on the rate of cell regeneration, and we may mention the fact that Neudörfer (10), one of the first to describe normoblast crises, observed them in cases of chlorosis.

A further question which naturally arises in relation to these sudden floods of nucleated red cells is: What is their fate? They disappear as quickly as they appear. As far as we know, their fate is entirely unexplained. Zuntz and his associates (11) believe that they are destroyed if present freely in the blood stream, but do not explain how. Our own observations, in which we have seen them resist the pounding of a mechanical glass perfusion pump through many hours without reduction in number, lead us to believe that they are as resistant to trauma as are non-nucleated cells.

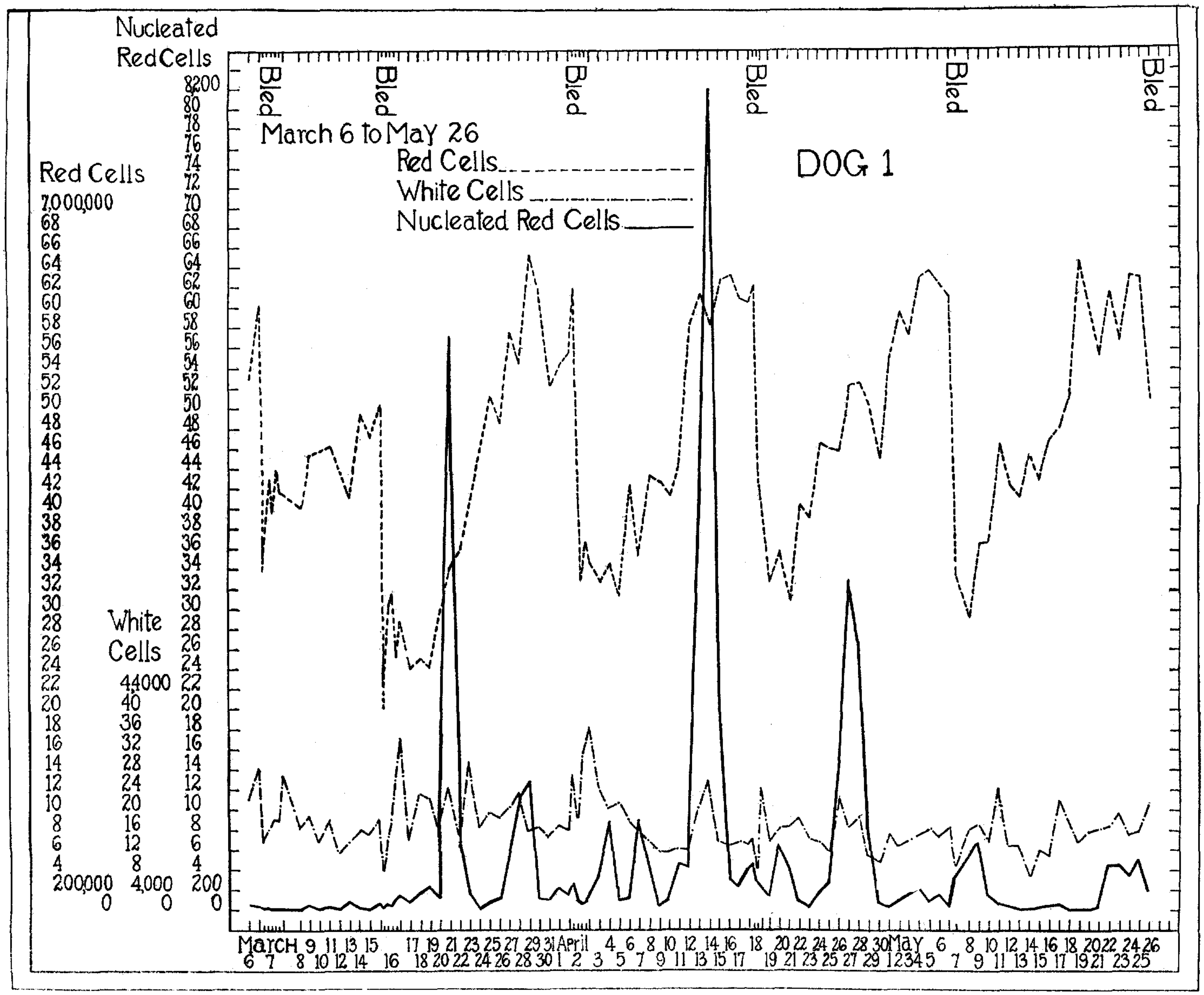
Concomitant Behavior of Red and of White Cells.

Those who have watched blood regeneration closely have been impressed with a tendency towards simultaneous movement of red and of white cells from the marrow. A high, sudden leukocytosis, polymorphonuclear in character, is frequently accompanied by a few nucleated red cells. Hough and Waddell (12) in studies of regeneration from single large hemorrhages in dogs state:

> "Each rise in the erythrocyte count is accompanied by a distinct rise in the leucocyte count either on the same or the preceding day. It is believed that, in general, when other causes of leucocytosis are controlled or absent, the leucocyte count may be taken as an indication of the degree of activity of the blood forming organs."

They do not report upon stained films, however, and have evidently observed increases in leukocyte count such as frequently accompany or just precede blast crises in our experiments. In the case of Dog 3, Text-fig. 1, the curves of nucleated red cells and leukocytes follow one another closely. Dog 4, Text-fig. 2, also shows increases in leukocyte count just before or with the blast crises of March 1 and 21 and April 9. However, during the period from April 12 to June 6, in which three large hemorrhages are given and recovered from, there are no normoblast crises, and with one exception no marked leukocytoses except those immediately after the hemorrhages. On April 24, 25, and 26 a definite leukocytosis occurs, caused by a localized abscess in the neck wound made in the bleeding on April 17. The white cells in this case have appeared in increased numbers and independently of normoblasts. Dog 1, Text-fig. 3, shows insignificant leukocytic increases with the blast crises, but nothing which alone would be significant as pointing to a regenerative tendency except possibly the leukocytosis beginning on April 13 and ending on the 15th. We may therefore agree with Hough and Waddell in feeling that a sudden and unaccountable leukocytosis may foretell rapid regeneration, but the reaction by no means always occurs before regeneration. Indeed, a tendency to avoid leukocytosis in the typical crisis position in the blood regeneration curve occurs as marrow hyperplasia advances. That the animals are well supplied with leukocytes is indicated by the vigorous posthemorrhagic leukocytoses which are seen to follow the hemorrhages throughout the entire course of the observations, and also from the readiness of development of the infectious leukocytosis in Dog 4.

Lastly, we should mention briefly posthemorrhagic leukocytosis. We have been impressed by the reliability and the extreme rapidity with which this reaction comes on. We have frequently seen the white count double in 2 hours. This is not a change in distribution of the white cells, since we have found the increase in specimens taken at the same time from heart, vein, and capillary blood. It is a real increase in the number of circulating white cells, and emphasizes the fact that the marrow is a reservoir of leukocytes in contrast to its relation to the red cells. Adult red cells are extruded minute by minute as fast as they are formed, and there is every tendency to



TEXT-FIG. 3. Curve of the daily red cell, white cell, and nucleated red cell counts of Dog 1 during a period of 3 months, showing the immediate and subsequent effects of six hemorrhages. Owing to the extremely large true blast crises which occurred in this animal the scale used in indicating the number of normoblasts is one-half the scale used for Dogs 3 and 4. The chief features in this curve are the same as those in Text-figs. 1 and 2.

resist undue haste in extrusion, as shown by the increasing power to hold nucleated cells which develops after a certain number of hemorrhages. But the marrow has an emergency function in relation to the leukocytes, a capacity to discharge the cells independently and rapidly, at times when many normoblasts are present, and under the influence of a procedure—hemorrhage—which brings about gradual increase in red cell counts.

Numerical Overregeneration of Red Cells and Influence of Repeated Hemorrhages on Rate of Regeneration.

Boycott and Douglas (8) have shown that after repeated bleedings in rabbits the rate of regeneration of hemoglobin becomes much greater than after a single hemorrhage, and that overregeneration occurs after single and multiple hemorrhages. The phenomenon of overregeneration of cells appears in our experiments. The high points in the curves become higher and higher as one passes to the right across the charts. This is particularly well seen in Dog 3, but in our opinion is equally true of Dog 4. The initial high red cell count in the latter animal was probably a fright polycythemia such as has been described by Lamson (13). The final regeneration after the bleeding was interrupted on June 6 by the utilization of this dog in a marrow perfusion experiment. Were it not for the fact that Boycott and Douglas have shown that there is a needless overregeneration of hemoglobin after hemorrhage, one might think that the polycythemic tendency which the repeatedly bled animals have shown was certainly due to the lag in hemoglobin formation, a multiplicity of cells carrying small amounts of hemoglobin taking the place of fewer cells better supplied. We followed this posthemorrhagic polycythemia for 2 months after the last bleeding in one animal, No. 2, upon whom, after six hemorrhages, all bleedings were discontinued. The red cell count at the end of this time was 9,336,000 cells per c.mm. This persistent polycythemia after repeated hemorrhages is comparable with the slow disappearance of the polycythemia of low barometric pressure. Apparently marrow hyperplasia once established resists disestablishment to a marked degree.

In considering the tendency to regenerate without crises, an adaptation of great interest, we have not met the criticism that such a process may be due to marrow exhaustion. In all three animals whose records are given, autopsy with subsequent histological examination of the marrow disclosed marked hyperplasia, extension of erythrocyte-bearing marrow into the shafts of the long bones, and many islands of erythroblastic activity. Further, if one takes 6,000,000 cells per c.mm. as the normal red cell count of these animals, it is seen that regeneration to this level takes place in almost the same length of time throughout the entire series of bleedings. It is noteworthy that there is no significant increase in speed of regeneration as marrow hyperplasia advances, but that at the same time there is no lag in red cell formation. It has not been possible to confirm the work of Boycott and Douglas (8) on increased speed of hemoglobin regeneration by an analogous increase in speed of red cell regeneration. We have shown that after successive hemorrhages regeneration is not delayed, that the process apparently becomes more orderly as evidenced by the failure to find normoblasts in the peripheral blood, and ultimately reaches an abnormal height. Boycott (14) notes the fact that young rats and rabbits with hyperplastic marrow do not extrude nucleated red cells after hemorrhage as do adults. Our experiments with dogs do not entirely verify this since one of our animals, No. 1, a pup weighing 5.2 kilos when first obtained and 8.6 kilos at death $2\frac{1}{2}$ months later, showed large crises of normoblasts when first bled and passed into the state of regenerating without crises in much the same manner as did the adult dogs.

CONCLUSIONS.

1. Hemorrhage with immediate saline infusion causes the appearance in the peripheral blood of a slightly increased number of normoblasts provided normoblasts are already present in the blood stream. Marrow hyperplasia does not intensify this reaction and the cells found probably do not leave the marrow pulp but are in the blood stream at the time of the experiment.

2. The slight increase in cells occurring immediately after hemorrhage and infusion is designated a pseudocrisis. True crises are much more extensive; they tend to occur just before rapid increase in the erythrocyte count and usually towards the end of the 1st week following hemorrhage.

3. Red cells and white cells tend to move from the marrow together, but this association is not invariable.

4. After repeated hemorrhages regeneration occurs independently of the appearance of nucleated red cells in the peripheral blood.

5. Repeated hemorrhages associated with extension of erythrocyte-producing marrow lead to polycythemia but not to a conspicuous increase in speed of regeneration.

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