THE INFLUENCE OF INORGANIC IRON ON THE REGENERATION OF BLOOD AFTER HEMORRHAGIC ANEMIA *

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The controversy over the therapeutic effect of inorganic iron has raged for several centuries, in fact, very considerably has it occupied the minds of medical men since Menghis, in 1746, reported that he had found iron in the blood of man. This controversy continued up until the past fifteen years, when Abderhalden, in 1906, abandoned his contention that iron could not be converted into hemoglobin. Since, then, it has been very generally accepted that inorganic iron is converted into hemoglobin as is the iron in the food and the so called organic iron. A re-opening of this controversy might be said to have occurred last year, when Whipple¹ and his co-workers published their studies on the regeneration of the blood after anemia. In one of this series of papers they show that ordinary inorganic iron in the form of Bland's pills has little or no effect on blood regeneration without adequate diets, and they are lead to draw the conclusion that "we may not assume, without positive proof, that inorganic iron is of value in the treatment of simple anemia."

The present series of experiments were undertaken for the purpose of determining whether this conclusion would hold true in the type of anemia that represents more closely the type that is seen in clinical medicine than that which occurs after a single large or massive hemorrhage. The opportunity was presented of securing some animals which had repeatedly been deprived of small amounts of blood over various intervals of time, and which had thus been rendered anemic. The anemia that these animals showed represented very truly the type of anemia which occurs after recurring loss of small amounts of blood and which the physician is called on most frequently to treat. These animals had been bled for varied lengths of time over periods ranging from six to eight weeks, and then immediately before starting the use of iron, they were rendered more anemic by one or more large bleedings. It is, of course, recognized that the factor of damaged blood formation (which is more or less of a factor in many clinical anemias) was not included in either these or Whipple's experiments on the efficacy of inorganic iron.

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1. Whipple, G. H., Hooper, C. W., and Robschey, F. S.: Blood Regeneration following Simple Anemia, *Am. J. Physiol.* **53**:263, 1920.

Fig. 1.—Hemoglobin and erythrocytes of Dog 19-4 and control.

Fig. 2.—Hemoglobin and erythrocytes of Dog 20-12 and control.

METHODS

In order to follow as carefully as possible the response of the animal to repeated hemorrhage, the following studies were undertaken: hemoglobin estimation, red cell count, enumeration of the white blood cells, differential count, resistance of the red cells to hypotonic salt solution, occasional observation on the percentage of skein cells and the determination of the total blood volume. The hemoglobin was estimated by the method of Newcomer; the red cells and white cells were counted by the usual methods on the Levy counting chamber; the resistance of the red cells was determined by the addition of a known quantity of red cells to a known quantity of hypotonic salt solution in ascending proportions, ranging from 0.25 to 0.5 per cent.; the skein cells were stained vitally with brilliant cresyl blue in potassium oxalate and counted with the oil immersion lens; and the blood volume was estimated by the method of Keith, Rountree and Geraghty. The blood for counting was collected from a puncture in the ear vein, made at approximately the same time each day and always before the animal had been fed. The blood for the volume estimation was taken from the jugular vein. The bleeding of the animals was from the jugular vein and in various quantities. The animals selected were apparently healthy, young adult dogs, and in order that the factor of diet might more closely approach that of the normal and bear no relationship to anemia, they were fed on the standard diet for all the dogs in the kennels, which consisted largely of hospital scraps. The animals were healthy throughout the course of the experiment and most of them gained weight. The blood counts were made at the start of the experiment and subsequently every second or third day. Except in one experiment, the dog which was given the iron and the control dog were studied the same day.

The iron was given in the form of equal parts of ferrous sulphate and sodium bicarbonate in capsule. This combination of iron presents the iron carbonate in a soft and powdery form. The capsule can readily be placed in the dog's mouth and pushed down with the finger until the dog swallows it. The gelatin capsule dissolves rapidly, and in such a form that it would not pass through the intestines undissolved as do the tightly compressed Bland tablets at times. The dose of iron given each dog was estimated on the basis that an average dose for a human being of 65 kg. weight is 1 gm. a day. A proportionate dose was worked out for each dog according to his body weight on commencing the experiment. In order to make sure that sufficient iron was given, this dose was doubled; that is to say, an amount of iron equivalent to 2 gm. per diem for man, was given each dog.

EXPERIMENTAL DATA

Experiment 1.—In the first experiment, two dogs were employed which had been a long time splenectomized and which had been rendered anemic as the

Fig. 3.—Hemoglobin and erythrocytes of Dog 20-11 and control.

Fig. 4.—Hemoglobin and erythrocytes of Dog 20-4 and control.

result of weekly bleedings of 100 c.c. for a period of two and one-half months. Following the preliminary study, the two animals were bled respectively 400 c.c. The amount of blood taken from both animals was approximately the same. During a period of nearly two months blood counts were made, as a rule, every second or third day with occasionally three days intervening between counts. During this interval of time it can be noted that the dog which was given iron following the preliminary hemorrhage, 19-4, returned more rapidly than did the control dog, 19-6, to a higher hemoglobin estimation and, likewise the

TABLE 1.—DOG 19-4. LONG TIME SPLENECTOMIZED. BLED 100 C.C. A WEEK FOR 2½ MONTHS. CONTROLLED BY 19-6.

Date	Hb.	R. B. C.	Resistance	Sk.	Blood Volume	Weight
6/28	85	4,640	26-38	0-400		13,450
6/30	Bled, 430 c.c.	3,520	26-38			
7/ 1	Start iron					
7/ 2	63	3,770			T. V. 950 T. P. 740 T. R. 210	13,250
7/ 4	68	4,040				
7/ 6	70	4,770	26-38	4-100		13,240
7/ 8	72	4,970				
7/10	74	4,820				
7/12	78	5,240	28-38	2-100	T. V. 1,300 T. P. 757 T. R. 543	13,200
7/14	83	5,810				
7/16	83	5,670	26-38			
7/19	81	5,680				
7/21	78	5,470	28-40	1-200		
7/23	78	4,750			T. V. 1,042 T. P. 687 T. R. 355	
7/26	82	5,360				
7/27	86	5,160				
7/30	87	5,910	30-44			
8/ 2	90	5,630			T. V. 1,400 T. P. 895 T. R. 505	
8/ 6	79	5,670				
8/10	79	5,890	30-40		T. V. 1,140 T. P. 752 T. R. 388	13,260
8/13	83	5,720				
8/16	86	5,650	28-40	1-200		
8/20	90	6,130			T. V. 1,220 T. P. 756 T. R. 464	
8/24	95	6,040	26-40	1-500		
8/26	91	6,010			T. V. 1,175 T. P. 753 T. R. 422	13,370

number of red cells was increased more rapidly (Tables 1 and 2). However, dog 19-6, the control animal, started with a decidedly lower hemoglobin percentage than did dog 19-4, while in the course of the experiment both animals arrived about the same time at the high water mark in their counts. The resistance of both animals to hypotonic salt solution was about the same and varied somewhat from day to day. The marrow response, as shown by the skein cells, was somewhat more pronounced in the dog which was receiving the iron. The blood volume showed in the dog which was the more anemic, the greater reduction at the start of the experiment, and this continued about the same through the length of the experiment. The last volume estimation, however, did show, in spite of the fact that the volume of 19-4 was higher than 19-6, that the total-red cells of this latter dog were considerably higher. During

the course of the experiment it is to be noted that dog 19-4, which was taking the iron, developed an acute drop in the number of red cells and percentage of hemoglobin, as well as in the total blood volume, without any demonstrable cause. From this causeless short period of increased anemia the animal rapidly returned to its former standard.

Experiment 2.—The second experiment was analogous to the first, except that the dogs employed had not been splenectomized. Dog 20-12 had been bled steadily for a period of eight weeks, in quantities of about 150 c.c. every week.

TABLE 2.—DOG 19-6. LONG TIME SPLENECTOMIZED. BLED 100 C.C. A WEEK FOR 2½ MONTHS. CONTROL OF 19-4.

Date	Hb.	R. B. C.	Resistance	Sk.	Blood Volume	Weight
6/28	68	4,450	28-40	0-400		14,000
6/30	Bled, 400 c.c.	3,640	26-38			
7/ 2	..	3,750			T. V. 845 T. P. 660 T. R. 185	13,880
7/ 4	65	3,560				
7/ 6	63	3,790	26-40	5-100		13,050
7/ 8	59	4,100				
7/10	61	4,430				
7/12	69	4,560	24-38	1-500		
7/14	66					
7/16	64	4,890	28-36		T. V. 1,035 T. P. 756 T. R. 279	13,430
7/19	65	4,970				
7/21	65	4,800				
7/23	62	4,996	26-38	1-400		
7/26	70	4,960			T. V. 970 T. P. 650 T. R. 320	13,600
7/27	71	5,060				
7/30	71	5,260	26-42			
8/ 2	79	5,290			T. V. 1,150 T. P. 758 T. R. 392	
8/10	76	5,870	26-38	1-400	T. V. 1,158 T. P. 730 T. R. 428	13,130
8/13	80	5,940				
8/16	83	6,210	24-38	1-400	T. V. 1,030 T. P. 628 T. R. 402	
8/20	86	6,070				
8/24	92	6,160	24-38	1-500	T. V. 1,070 T. P. 643 T. R. 527	13,300
8/26	86	6,080				

The control dog had been bled almost steadily for seven months. At the beginning of the experiment, the two dogs had relatively the same hemoglobin percentage. Dog 20-12, however, had a red cell count of a million and a half less than the control dog, 19-3 (Tables 3 and 4). The control dog was also the much larger animal, with red cells which were more resistant than in the other animal. The blood volume of the two dogs was about the same on commencing. However, the control dog's subsequent estimation showed a much larger total volume. While the red cells were absolutely greater in number in this animal than in his fellow, the much larger plasma content would indicate a relatively lessened concentration of the erythrocytes in the peripheral circulation as well as a lower hemoglobin estimation, so that in this animal the blood volume estimation shows that the control dog is regenerating more rapidly and more

TABLE 3.—DOG 20-12. BLED 150 C.C. WEEKLY FOR 8 WEEKS, LAST BLEEDING 6-28. CONTROLLED BY 19-3.

Date	Hb.	R. B. C.	Resistance	Sk.	Blood Volume	Weight
6/28	53	4,016	30-42	10-300		9,510
7/ 1	Start iron				T. V. 967	
7/ 2	58	4,650			T. P. 725	9,950
					T. R. 242	
7/ 6	57	5,400	30-44	2-100		
7/ 8	69	5,440				
7/10	74	6,080				
7/12	74	6,760	28-46	1-200		11,000
7/14	71	6,850				
7/16	78	6,980	30-44		T. V. 850	10,360
					T. P. 552	
					T. R. 298	
7/19	75	6,880				
7/21	80	7,170				
7/23	83	7,100	30-44		T. V. 796	11,400
7/26	88	7,290			T. P. 478	
					T. R. 318	
7/28	84	7,650	28-44		T. V. 1,040	11,000
7/30	84 stop iron	7,990			T. P. 655	
					T. R. 385	
8/26	101	7,970			T. V. 1,235	11,200
					T. P. 655	
					T. R. 580	

TABLE 4.—DOG 19-3. BLED 100-150 C.C WEEKLY FOR 7 MONTHS, LAST BLEEDING 6-28. CONTROL OF 20-12.

Date	Hb.	R. B. C.	Resistance	Sk.	Blood Volume	Weight
6/28	55.5	5,500	26-36	0		15,730
7/ 2	62	6,420			T. V. 995	15,560
					T. P. 735	
					T. R. 260	
7/ 6	66	6,060	26-38	1-200		15,440
7/ 8	78	6,380				
7/10	83	6,570				
7/12	82.5	6,440	24-38	1-200		
7/14	83	6,880	26-38			15,660
7/19	83	7,040				
7/21	81	7,090				
7/23	82	7,130	24-36		T. V. 1,234	14,790
7/26	88	7,270			T. P. 740	
					T. R. 494	
7/28	92	7,880			T. V. 1,394	
7/30	89	7,530	24-40		T. P. 850	
					T. R. 544	
8/26	90	7,520			T. V. 1,332	15,140
					T. P. 800	
					T. R. 532	

TABLE 5.—DOG 20-11. BLED 900 C.C. IN SMALL BLEEDINGS, IN TWO MONTHS. THEN 100 C.C. EVERY OTHER DAY FOR THREE DAYS, FOLLOWED BY A BLEEDING OF 350 C.C. CONTROLLED BY 20-13.

Date	Hb.	R. B. C.	Resistance	Sk.	Blood Volume	Weight
7/16	52	3,540	26-40	14-100		17,330
7/19	Start iron 64	3,970			T. V. 1,142 T. P. 812 T. R. 330	16,040
7/21	63	5,080	28-42	10-100		
7/23	70	5,490	28-42	6-100		
7/26	80	5,650	26-42	3-100		
7/28	86	6,320			T. V. 1,320 T. P. 885 T. R. 435	16,140
8/ 2	86	6,360	26-42	4-100	T. V. 1,340 T. P. 856 T. R. 484	17,690
8/ 4	86	6,920			T. V. 1,440 T. P. 864 T. R. 576	17,910
8/ 8	86	6,950	26-42	1-300	T. V. 1,398 T. P. 740 T. R. 658	
8/13	84	7,190				
8/17	98	8,040	28-42	2-500		
8/20	98	8,350				

TABLE 6.—DOG 20-13. BLED 600 C.C. IN SMALL BLEEDINGS IN 6 WEEKS. THEN DAILY BLEEDING OF 100 C.C. FOR THREE DAYS AND 250 C.C. ON EACH OF THE FOLLOWING THREE DAYS. CONTROL OF 20-11.

Date	Hb.	R. B. C.	Resistance	Sk.	Blood Volume	Weight
7/26	64	3,120	24-40		T. V. 813 T. P. 502 T. R. 311	15,600
7/28	59	3,250	26-40			
7/30	59	3,600	28-42	5-100	T. V. 1,026 T. P. 797 T. R. 223	15,510
8/ 2	63	3,630				
8/ 4	60	4,120	28-42		T. V. 1,225 T. P. 857 T. R. 368	
8/ 8	72	4,010	28-42	1-400	T. V. 1,270 T. P. 825 T. R. 445	15,550
8/13	73	4,970	26-40	1-600	T. V. 1,200 T. P. 722 T. R. 478	
8/17	86	5,860	28-38	4-400	T. V. 1,560 T. P. 845 T. R. 715	15,810
8/20	92	6,740				
8/23	100	7,800	28-40	1-400		
8/26	101	7,650				

completely than the simple peripheral counts would indicate. The control dog, in the month that the counts were being made, recovered from his anemia rather rapidly, as did the other dog. At the end of the month, the hemoglobin of the control dog was slightly higher, while the red cells were slightly lower, than the other animal. The blood volume of the larger dog, that is the control, had increased, as was to be expected, very much more rapidly than the other animal, and the same holds good in the total number of red cells. At the end of the month the iron was stopped. Nothing further was done to the dogs until a month later, when they were again counted. The count at this time showed that the dog which had been receiving the iron, had increased its hemoglobin percentage to 101, and apparently had not recovered from its anemia when the

TABLE 7.—DOG 20-4. LONG TIME SPLENECTOMIZED. CONTROLLED BY 20-5.

Date	Hb.	R. B. C.	Resistance	Sk.	Blood Volume	Weight
8/ 2	92	5,190				10,000
8/ 3	Bled, 350 c.c.					
8/ 4	44 Bled, 175 c.c. start iron	2,980	26-40			10,100
8/ 6	41	3,010	28-42	1-100		
8/10	66	4,040	28-40	4-100	T. V. 895 T. P. 597 T. R. 298	
8/13	71	4,620				11,500
8/16	78	4,780	28-42	2-100		
8/20	86	5,110			T. V. 940 T. P. 563 T. R. 377	
8/23	92	5,520	28-42	1-100		12,400
8/27	96	5,680				

TABLE 8.—DOG 20-5. LONG TIME SPLENECTOMIZED. CONTROL OF 20-4.

Date	Hb.	R. B. C.	Resistance	Sk.	Blood Volume	Weight
8/ 2	93.5	5,120				16,000
8/ 3	Bled, 425 c.c.					
8/ 4	50 Bled, 350 c.c.	3,070	30-42			16,600
8/ 6	42	3,100	32-44			16,800
8/10	63	3,650	28-42	3-100	T. V. 1,065 T. P. 756 T. R. 309	16,730
8/13	71	3,910				
8/16	79	4,750	28-42	4-300		16,800
8/20	92	5,740				
8/23	98	5,690	28-40	2-300	T. V. 1,320 T. P. 738 T. R. 582	16,900
8/27	102	5,980				

count had been stopped a month earlier; whereas, with the control dog, figures that were normal for that dog had been reached when the experiment was stopped. It is interesting to note that throughout the course of this experiment the resistance of the control dog was always greater than the animal which was receiving the iron.

Experiment 3.—The procedures in this experiment were altered somewhat. Two animals, 20-11 and 20-13, which had been bled small quantities every six or eight weeks, were then bled 100 c.c. for three consecutive days, and then larger bleedings for the next three days. When these bleedings were stopped, the dog which was to receive the iron, 20-11, had a lower hemoglobin estimation and a higher red cell count than did the control dog (Tables 5 and 6). This dog

also showed a very vigorous marrow response, as shown by the skein cells. The total blood volume was considerably higher than with the control. The severity of the anemia is shown by the blood volume estimations to be much greater than the peripheral blood counts would indicate. Regeneration of the red cells after the hemorrhage was more rapid in the dog taking the iron. On the other hand, in the control dog, the hemoglobin rose slowly but ultimately attained higher figures than it did with the iron-taking animal. In this experiment the resistance of the red cells to hypotonic salt solution was slightly greater in the control animal. The skein cells in the animal receiving the iron were more frequent during the course of the experiments than in the animal that was used as a control. To return again to the blood volume, it can be seen that the total volume of the iron taking dog, the heavier of the two, remained greater than in the control dog until the last count. Likewise, the total reds of the control dog were considerably higher at the end of the experiment than in the test dog. Both animals increased in weight during the course of the experiment.

Experiment 4.—The last experiment was undertaken for the purpose of determining the rapidity of the blood regeneration of two splenectomized animals who had been splenectomized a long time. Both animals, 20-4, and 20-5, were relatively anemic at the start of the experiment (Tables 7 and 8). Each animal was bled as large a quantity as could be taken from him on two successive days and both were rendered extremely anemic. The anemia of the control dog, the smaller of the two, was greater than that of the other after the hemorrhages. Both dogs returned, in a period of a little over three weeks, to a higher standard than before the bleeding. The resistance of the red cells in the two animals during the course of the experiment was about the same. The skein cells showed a somewhat more active marrow response in the iron taking animal. In this latter dog, the blood volume was also greater as were the total reds. Both dogs gained weight.

DISCUSSION

The results of these experiments would seem to substantiate the contention of Whipple that under ordinary circumstances the giving of inorganic iron does not have a particularly marked effect on blood regeneration and the correction of hemorrhagic anemia in experimental dogs. It may be contended, and, we believe, rightly so, that the return to the normal in these animals is so rapid that the iron can have but little effect when nature is taking care of the anemia so thoroughly. However, in two of the dogs, which had been rendered anemic by repeated bleedings and by a long time splenectomy, the return to normal was relatively slow, yet in neither the control, 19-6, or the experimental dog 19-4 was there any obvious or material difference in the capacity of the regeneration of the red cells or hemoglobin. Difference, of course, exists between the dog taking iron and the controls, but only such as would be expected. It would be almost impossible to obtain animals which were alike in every respect. The hematopoietic systems of no two animals will respond in exactly the same manner. In considering experimental work of this type, various factors must be taken into consideration. The resistance of the red cells to hypotonic salt solution in some animals is greater than in others, and this must play some factor in altering the course of anemia. In

some animals, the response of the marrow and the stimulation of hemorrhage is greater than in other animals. This can be demonstrated by the skein cells. In some animals the blood volume is greater and better sustained than in others. Lastly, there is no absolute mean normal count as these and other factors product differences in the hemoglobin and red cells count in the different dogs.

SUMMARY

After hemorrhagic anemia in otherwise normal dogs, under standard conditions, dogs receiving iron in two of four experiments (Experiments 1 and 3), regenerated blood more rapidly than their controls during the early stages: but both test and control dogs attained figures approximately normal in about the same time interval. In the other two studies, regeneration is seen to be more rapid and complete in the control dogs, particularly when the blood counts are analyzed in relation to the blood volume changes.

CONCLUSION

The administration of inorganic iron cannot be said to produce any constant alteration in the course of an experimental hemorrhagic anemia.