A STUDY OF EXPERIMENTAL ANEMIA IN DOGS: THE ACTION OF BEEF LIVER AND IRON SALTS ON HEMOGLOBIN REGENERATION

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(Received for publication September 6, 1927)

Recent contributions to the study of hemoglobin regeneration in secondary anemia have laid stress on the therapeutic value of natural food products (1) (2). These reports have led to the indiscriminate administration of glandular organs, notably liver, to patients who had some form of secondary anemia.

The present investigation was undertaken in order to determine the exact rôle of iron salts in hemoglobin formation in long-standing experimental anemia, and to study the effect of feeding beef liver on hematopoietic activity. The exhaustive and well-controlled experiments of Whipple and his associates have advanced our knowledge greatly. However, the use of a wide variety of food products and the attempts to control the metabolism of pigment, and blood cell stroma, have introduced an element of uncertainty with regard to the quantitative effect of iron in bone marrow activity.

In experimental work of this type the factor of relative depletion of the body iron reserve cannot be over-emphasized. An adequate vitamin intake must be assured in order to maintain a healthy bone marrow (3). In the matter of iron depletion, years of clinical observation have demonstrated that in prolonged hemorrhage, from whatever cause, the administration of some form of iron is directly beneficial, without necessarily having a specific stimulating effect upon the bone marrow itself. Whipple and Robscheit-Robbins (2) have stated that the feeding of liver had a well-marked effect upon hemoglobin regeneration. In attempting to confirm this observation it seemed plausible to substitute for glandular organs an adequate supply of vitamin in the diet, and to study the effect of adding and withholding iron salts.

The general plan of study followed the methods of Whipple and Robscheit-Robbins, but several modifications will be described. Dogs were fed the basal bread mixture (C) (4), substituting for the salt mixture 500 cc. of whole milk to the daily ration. This insured the presence of an adequate amount of vitamin B. Facilities were not available to determine the iron content of the diet, but it was probably quite low. Preliminary experiments were carried out by feeding the original bread mixture of young rats previously deprived of vitamin B. When the bread was fed, the resulting growth curves showed a definite lag in the weights of the animals, as compared with the controls. The addition of milk had a further advantage for the animals consumed the bread mixture completely.

Plasma volume estimations were omitted, because the dogs were bled at intervals of at least two days; this is considered ample time for readjustment of the blood volume following moderate hemorrhage. The amount of blood withdrawn each time depended upon the hemoglobin percentage found at the time of the previous bleeding. After some experience, the size of the hemorrhage necessary to keep the hemoglobin level at about 50 per cent could be estimated. The amount of blood withdrawn varied from 30 cc. to 300 cc. The small samples of blood taken between the larger bleedings were helpful in determining the rate of hemoglobin regeneration and in deciding upon extent of the succeeding hemorrhage. As the protocols show, the larger hemorrhages were produced at fairly long intervals.

Hemoglobin determinations were made by the oxygen capacity method of Van Slyke and Stadie, the value of 18.5 volumes per cent being used as a standard, with 13. 8 grams of hemoglobin per 100 cc. of blood. Red blood cell counts and reticulated cell counts were made by standard methods when indicated. Blood was drawn under oil from the femoral arteries, without exposing the vessel and without stasis, dry potassium oxalate being used in just sufficient quantity to prevent coagulation (0.2 per cent).

The periods of experimental feeding were usually ten days in duration when liver, thymus, and commercial nucleic acid were used. An observation period of twenty days was usually allowed in which to note the latent effect of the food upon hemoglobin regeneration. Iron was administered usually in the form of ferric citrate in amounts

of 0.8 gram daily, dissolved in water and mixed with the diet. This amount was chosen in order to provide a definite excess of the mineral without producing toxic symptoms. In two experiments ferrous carbonate was used. In contrast to the method of feeding liver, the administration of iron salts was continuous throughout the experimental period.

The experiments were planned to show the effect on hemoglobin regeneration of (1) adequate iron intake in combination with liver, (2) liver feeding in the presence of iron depletion, and (3) the effect of iron salts alone.

TABLE 1

Rate of hemoglobin regeneration when iron was added to the basal bread mixture

Dog number	Days	Added to diet	Average weight of dog	Hemoglobin regenerated		
				Total grams	Grams per day	Grams per kilogram of animal per day
			kgm.			
8	38	Ferric citrate	13.63	84.0	2.21	*0.162*
3	40	Ferric citrate	12.72	68.8	1.72	*0.135*
69	35	Ferric citrate	15.23	82.5	2.357	*0.155*
72	25	$FeCO_3$—0.6 gram	15.05	47.0	1.88	*0.121*
4	47	Ferric citrate	12.8	101.0	2.149	*0.175*
70	37	Ferric citrate	14.4	81.9	2.213	*0.153*
9	26	Ferric citrate	11.8	48.0	1.843	*0.156*

RESULTS

Table 1 shows the results observed when ferric citrate was added to the basal bread mixture. In these experiments the animals had previously been bled over a long period of time while being fed the basal diet, and relative iron depletion was present at the beginning of the experiments.

Table 2 shows the effect of adding 400 grams of raw beef liver to the diet each day for ten days. A period of twenty days was found to be sufficient for the effect of liver feeding to manifest itself, and this period was added to the feeding period (except in one case), making the total time for the experiment thirty days.

When liver was fed, a relation was apparent between the available

body iron (or relative iron depletion), and the rate of regeneration of hemoglobin. Referring to table 1, it will be seen in the experiment with dog 4, that when ferric citrate was used alone, the rate of regeneration of hemoglobin was 2.149 grams per day. While ferric citrate was being continued, the dog was fed 4,000 grams of raw beef liver during a period of ten days, and hemoglobin was recovered at the rate of 2.213 grams per day (table 2). No specific stimulation is apparent. In the experiment with dog 69, (table 2), 400 grams of liver per day were fed, beginning twelve days after the preliminary bleedings had started. This allowed little time for the depletion of the body iron reserve. When liver was added to the diet, the amount of hemoglobin recovered was 2.533 grams per day, and in a later

TABLE 2

Rate of hemoglobin regeneration when raw beef liver was added to the diet

Dog number	Days	Added to diet	Average weight of dog	Hemoglobin regenerated		
				Total grams	Grams per day	Grams per kilogram of animal per day
			kgm.			
69	30	400 grams. 10 days	13.2	76.0	2.533	*0.1919*
4	30	400 grams. 10 days	13.0	66.4	2.213	*0.1702*
3	18	400 grams. 10 days	11.7	24.5	1.36	*0.1152*
70	30	400 grams. 10 days	14.4	48.3	1.61	*0.1118*
9	30	400 grams. 10 days	11.8	47.8	1.593	*0.135*

experiment with iron alone, 2.351 grams per day was recovered. In the three experiments in table 2 in which relative iron depletion did precede the administration of liver (dogs 3, 70, and 9), the resulting regeneration of hemoglobin was somewhat greater than with the basal diet mixture alone, but *definitely less* than when iron was abundantly provided. In an animal given a series of rapid preliminary bleedings to reduce the hemoglobin level to 50 per cent, the rate of regeneration *immediately* following is greater than that caused by either liver feeding or iron administration. This is shown by an experiment with dog 8, in which the regeneration rate was 3.434 grams per day in a three weeks period, which had begun two weeks after the initial hemorrhage. (See protocol.) These observations illus-

trate the profound effect of the condition of the body iron reserve on experiments of this nature. Thus, when the iron reserve was high the hemoglobin regeneration was quite pronounced, both on the basal diet and when liver was added, but when the iron reserve was low, the hemoglobin regeneration could be increased to maximum by supplying iron salts alone.

Experiments were made to determine the efficacy of chlorophyl, wheat germ oil, and commercial yeast nucleic acid. The nucleic acid

TABLE 3

Dog number	Grams of hemoglobin regenerated per kilogram of dog per day		
	Nucleic acid added to diet	Raw thymus gland added to diet	Raw liver added to diet
	grams	*grams*	*grams*
3	0.250	0.2041	0.1152
69	0.125		0.1919
4	0.258		0.1702
70	0.1846		0.1118
2		0.2460	

TABLE 4

The effect of the bread and milk mixture alone on the rate of hemoglobin regeneration

Dog number	Days	Average weight of dog	Hemoglobin regenerated		
			Total grams hemoglobin	Grams per day	Grams per kilogram per day
3	30	12.6	41.6	1.386	*0.11*
72	28	14.5	38.3	1.367	*0.094*
4	32	12.3	28.0	0.875	*0.071*
9	25	12.36	27.5	1.10	*0.089*

was fed in moderate amounts (15 to 20 grams per day) for periods of ten days, and in calculating its effect a "carry-over" period of twenty days was allowed. The results are about equal to those derived from liver and the feeding of thymus gland; but only when iron was constantly supplied in the diet was the regeneration of hemoglobin increased. Table 3 shows these in comparison.

In table 4 are shown the results of feeding the basal bread mixture alone to dogs in which there was relative iron depletion. The diet

was not entirely iron free, and we have available only indirect data to show the amount of iron depletion in the animal's body. It is obvious, however, that the rate of hemoglobin regeneration is greatly depressed, when these results are compared with those in the preceding tables.

COMMENT

There was no evidence in these experiments to show that the feeding of beef liver is of especial value in anemia of long duration produced in healthy animals by hemorrhage. The conclusions to be drawn appear directly opposed to those of Whipple and Robscheit-Robbins, whose work this study has attempted to confirm. However, a fairly complete agreement may be secured in the conclusions by applying a different interpretation to the results of Whipple and Robscheit-Robbins. The latter investigators have appreciated the action of iron in experiments of this type, but apparently in their experiments there was not provided an adequate amount of iron in the animals' diets to control this factor and prevent possible iron depletion while administering natural food products. In drawing conclusions with regard to hemoglobin regeneration it seems that the body iron reserve must be fully protected if the effects of the iron content of the foods themselves are to be avoided.

According to Wells (5) the average human body contains about 3.2 grams of iron, of which 2.4 to 2.7 grams are in the blood. If we consider that the weight of a dog is one-sixth that of a man, then the iron content of the dog's body would be about 0.6 to 0.7 gram. In order to prevent the normal rebound of blood regeneration after hemorrhage, as described by Drinker (6), a very large amount of hemoglobin must be removed. An idea of the extent of the hemorrhage necessary to accomplish this may be obtained by a study of the protocols of dogs 8 and 72. In these animals about 180 grams of hemoglobin were removed in 1700 cc. of blood during 30 to 35 days before rapid regeneration ceased. This corresponds to the removal from their bodies of 0.57 gram of iron. If we grant a small intake of iron with the food and water during this period, there was present a definite depletion of the body iron reserve in the animals at the beginning of the experiments. In feeding 4,000 grams of beef liver the

amount of iron administered, according to the analysis of Forbes and Swift (7), would be 0.328 gram. The recovery of 50 grams of hemoglobin would remove 0.168 gram from the animal's body. If these values represent the average results, it seems impossible to recover the iron quantitatively when absolute iron starvation is present. The figures here quoted are conservative, and a proper conception of their significance is prerequisite to an appreciation of the conditions under which the experiments were carried out.

A sharp distinction must be drawn between cellular regeneration and hemoglobin formation in the bone marrow, and a knowledge of the number of reticulated red blood cells in the circulation is of fundamental importance in this respect. The reticulocyte percentages were surprisingly low, considering the large hemorrhages which were survived. This observation is susceptible of two interpretations: (1) either the response of the bone marrow was equal to the stress caused by the hemorrhages; or (2) the bone marrow was rendered relatively inactive by some such factor as faulty diet, or disease in the animal itself. It is obvious that the latter could not have been present, because after the preliminary bleedings the number of red blood cells in the circulation quickly returned to about the normal level, regardless of the dietary ingredients, and when iron was abundantly provided hemoglobin regeneration was not interrupted. It is evident that any definite stress placed upon the mechanism of healthy bone marrow to provide red blood cells would have been shown by a more pronounced increase in reticulocytes in the peripheral blood. For this reason, it must be assumed that the power of regenerating hemoglobin was alone being tested, and not the mechanism of cell production in the bone marrow.

A recalculation of the amount of hemoglobin regeneration in the experiments of Whipple and Robscheit-Robbins has been made on the basis of grams of hemoglobin regenerated per day per kilogram of animal. The figures are taken from the experiments in which cooked beef liver and Blaud's pills (2) (8) were used. In table 5 these recalculated figures are set down, together with the percentage of hemoglobin in the animals at the beginning, and at the completion of each experiment. It will be seen that in several of these experiments where the effect of liver feeding was sought, the animals lost

more than 25 per cent in the hemoglobin level during the progress of the experiment.

For illustration, referring to table 5, dog 19–104, in the first experiment 0.360 gram of hemoglobin was regenerated daily per kilogram of dog, but the animal lost 38 per cent in its hemoglobin level during the four weeks period. In another experiment on the same animal, the regeneration rate, as calculated from the records, is 0.142 gram, the loss in hemoglobin being 16 per cent. In the five experiments in table 5, in which Blaud's pills were administered during one-half

TABLE 5

Analysis of the results of Whipple and Robscheit-Robbins

Dog number	Days	Added to diet	Average weight of dog	Total grams hemoglobin regenerated	Grams hemoglobin regenerated per day per kilogram of animal	Hemoglobin level		
						Beginning of experiment	End of experiment	Difference
			kgm.			*per cent*	*per cent*	*per cent*
19–104	28	Liver	10.5	108.1	0.360	86	48	−38
19–104	28	Liver	11.0	43.7	0.142	52	36	−16
21–64	28	Liver	14.4	73.9	0.207	80	55	−25
16–160	28	Liver	10.8	36.4	0.120	57	48	− 9
20–104	28	Liver	9.6	56.3	0.209	58	45	−13
20–104	28	Liver	8.7	98.8	0.396	74	47	−27
20–71	28	Liver	16.0	64.1	0.144	48	50	+ 2
20–104	28	Blaud's pills	8.5	45.3	0.190	44	76	+32
20–71	28	Blaud's pills	11.7	35.6	0.108	53	43	−10
21–64	28	Blaud's pills	14.5	61.9	0.152	80	44	−36
24–3	28	Blaud's pills	12.1	35.2	0.105	51	44	− 7
24–2	28	Blaud's pills	12.3	41.4	0.120	44	46	+ 2

the experimental period, only three of the experiments are susceptible of interpretation, for the reasons given above. The results of the experiments in which the hemoglobin level remained at about a constant (table 5) are quite comparable with those recorded here, and no fundamental disagreement in conclusions need be drawn.

It must be evident then from the results of both sets of experiments that the feeding of liver had no demonstrable effect in the regeneration of hemoglobin in anemia of long duration in healthy animals, when there was an adequate intake of iron.

The conclusions of Whipple, Robscheit-Robbins, and Hooper (1),

regarding the effect of liver feeding proved successful fortunately, when applied to patients with pernicious anemia (9). The theoretical reason for this application is not clear, since the two conditions under which liver feeding must exert its action are totally different. The predominate cell of the bone marrow in hemorrhage is quite different from the predominate cell of pernicious anemia in relapse. The conditions imposed by these experiments upon the red blood cell production were at no time severe, and there was no evidence of delay in the development of red blood cells by the bone marrow, such as occurs in pernicious anemia. In these experiments, even under relative iron depletion the number of red blood cells tends gradually to approach normal. (See protocol, dog 3.) Thus, we have no experimental or theoretical evidence that the administration of relatively enormous amounts of liver to the dogs could have a favorable effect upon red blood cell production.

This paper has to do chiefly with hemoglobin regeneration, and further experiments are now in progress upon the phase of red blood cell regeneration.

I am greatly obligated to Dr. Sarah Long for her assistance in staining blood smears and making reticulocyte counts.

CONCLUSIONS

1. In the presence of an adequate diet, hemoglobin regeneration in the long continued experimental anemia of healthy adult dogs is limited by the availability of iron.

2. In this form of anemia the administration of liver has no specific action upon the regeneration of hemoglobin and acts entirely through its iron content.

3. Liver feeding caused no demonstrable increase in red blood cell production, such as occurs in pernicious anemia.

4. The therapeutic value of iron salts in the treatment of anemia resulting from hemorrhage is sustained.

5. A sharp distinction must be drawn between hemoglobin formation and cellular regeneration of the blood elements in this type of anemia.

6. Hemoglobin formation is depressed by relative iron starvation in long continued experimental anemia induced by hemorrhage, red blood cell production is somewhat less modified.

PROTOCOLS

Date	Weight	Hematocrit	Red cell counts	Hemoglobin level	Amount bled	Reticulocytes	Oxygen capacity	Hemoglobin	Experimental diet
Dog No. 70									
1927	*kgm.*	*per cent*	*millions*	*per cent*	*cc.*	*per cent*	*volumes per cent*	*grams*	
March 21	14.6			130	275	1.2		50	
March 23				111	260		20.5	40	
March 25				88.7	230		16.4	28	
March 28		20	4.496	65	200	3.2	12.1	18	
April 1		16	4.104	39	20	7.2	7.1	1.0	Yeast—nucleic acid—$FeCO_3$—0.6 gram
April 6		23	4.592	60	30	7.2	11.5	2.6	Yeast—nucleic acid—$FeCO_3$—0.6 gram
April 8		27	6.544	64	65	7.0	11.9	5.7	Yeast—nucleic acid—$FeCO_3$—0.6 gram
April 11	13.5	23	6.192	60	165	12.1	11.5	13.4	Yeast—nucleic acid—$FeCO_3$—0.6 gram
April 12									Diet—ferric citrate
April 15		29	6.304	62.7	180	13.2	11.6	8.1	Diet—ferric citrate
April 18		28	5.240	60	160	8.2	13.2		Diet—ferric citrate
April 20			5.808	59	150	7.8	11.0	12.8	Diet—ferric citrate
April 25			5.776	52	155	5.4	9.6	11.2	Diet—ferric citrate
May 2		20	6.928	55.7	40		10.3	3.0	Diet—ferric citrate
May 6	14.3	23	7.808	68.5	130		12.6	12.0	Diet—ferric citrate
May 11		30	7.408	70	220	5.6		21.0	Diet—ferric citrate
May 16	14.3	30	7.360	50	180	6.5	9.3	12.4	Diet—ferric citrate
May 20				62	160	6.2		13.0	Diet—ferric citrate
May 27			6.986	65	200	5.6		18.0	Diet—ferric citrate
June 1	14.5		7.840	60	30	5.1	11.1	2.5	Diet only
June 6		24	5.450	52	200		9.6	14.3	Diet only

June 8	13.8	26	6.904	47	30		8.7	2.0	Liver—400 grams
June 13			6.368	50.3	200		9.3	13.8	Liver—400 grams
June 20		26		49	215		9.1	15.4	Diet only
June 26			5.948	46.5	200		8.6	12.8	Diet only
July 4			5.216	45	100		8.3	6.2	Diet only
				Dog No. 8					
March 30	12.3	34		113.6	275		21	43	Diet only
April 4			5.776	82	225		15.3	25.6	Diet only
April 8		27		75	200			20.7	Diet only
April 11	12.8		4.840	55	200	7.4		15.2	Diet only
April 15		27	5.392	75.7	30	5.0	14	2.6	Diet only
April 18	13.6		4.816	76	130	3.8		13.6	Diet only
April 25			5.840	78	200	6.6		21.5	Diet only
April 27			5.632	66	155	2.2		14.0	Diet only
April 29			4.928	66	150	3.8		14.0	Diet only
May 2			4.256	53.5	110	6.3	9.9	7.4	Diet only
May 4	13.2	24	4.240	55.2	30	6.0	10.2		Ferric citrate—0.8 gram
May 9			5.336	54	100	4.0	10.0	7.4	Ferric citrate—0.8 gram
May 13			7.072	61			11.3	12.8	Ferric citrate—0.8 gram
May 17	13.6	26	6.032	60	200	5.6	11.0	16.4	Ferric citrate—0.8 gram
May 20				52	160			11.5	Ferric citrate—0.8 gram
May 24		27	6.720	61	100		11.3	8.5	Ferric citrate—0.8 gram
May 27			6.996	62	200	4.5		17.0	Ferric citrate—0.8 gram
June 1	13.6		6.864	55	30	4.2	10.1	2.2	Ferric citrate—0.8 gram
June 3				53	130		9.6	9.3	Ferric citrate—0.8 gram
June 8	13.6	28	6.816	54	115		10.0	8.5	Ferric citrate—0.8 gram
June 10				57	30		10.5	2.0	Ferric citrate—0.8 gram
June 20				51.4	100		9.5	7.0	Ferric citrate—0.8 gram
June 26			7.168	63.3	150		11.7	13.0	Ferric citrate—0.8 gram
July 4				62.5	215		11.5	18.0	Ferric citrate—0.8 gram
July 11			7.808	63.2	40		11.7	3.0	Ferric citrate—0.8 gram

PROTOCOLS—*Continued*

Date	Weight	Hematocrit	Red cell counts	Hemoglobin level	Amount bled	Reticulocytes	Oxygen capacity	Hemoglobin	Experimental diet
				Dog No. 69					
1927	*kgm.*	*per cent*	*millions*	*per cent*	*cc.*	*per cent*	*volumes per cent*	*grams*	
March 21	12.7			122	250	0.5		42	Diet only
March 23				92.8	240		17.2	30	Diet only
March 25				71	250		13.2	24.5	Diet only
March 28		20	4.992	59.5	180	7.0	10.9	14.8	Diet only
April 1		21	5.136	59.5	20		11.0		Liver 400 grams
April 6		24	5.616	72	140	7.7	13.3	13.9	Liver 400 grams
April 8		26		75	200			20.7	Liver 400 grams
April 11									Diet only
April 13	12.8	28	7.216	84	105	5.3	15.6	12.0	Diet only
April 16		28		73	130	3.8	13.5	13.0	Diet only
April 20				61	100			8.4	Diet only
April 25			6.192	68	85	3.2	8.0		Diet only
May 2		23	6.768	60	85	4.7	11.0	7.0	Diet only
May 6		21	6.816	68.5	130		12.6	12.0	Diet only
May 11	13.6		7.248	60	180	6.2			
May 13		23	5.680	45.7	80	7.4	8.45	5.0	Diet—nucleic acid—15 grams
May 16	14.8	25	6.320	47	200	18.7	8.7	13	Diet—nucleic acid—15 grams
May 20				50	200	12.1		15.8	Diet—nucleic acid—15 grams
May 25		25	5.360	43	170	14.2	8.0	10.2	Diet only
May 30		24	6.006	39	120	11.9	7.2	6.4	Diet only
June 6		23	5.460	41.7	30	5.5	7.7	2.0	Diet only
June 10				39.3	120		7.3	6.6	Diet only
June 20			7.888	45.4	120		8.4	7.5	Diet only

June 26			7.036	55.7	200		10.3	15.4	Ferric citrate
July 4				62.5	185		11.5	15.5	Ferric citrate
July 11			8.384	60.6	275		11.2	23.0	Ferric citrate
July 20				58	200		10.8	16.1	Ferric citrate
July 25				63	144		11.7	12.5	Ferric citrate
					Dog No. 9				
April 22	12.0		8.000	116	200	1.8	21.6	32.2	Diet only
April 25				86	225	2.0		32.2	Diet only
April 27			6.160	94	50			6.0	Diet only
April 29				82	170	6.7		19.6	Diet only
May 1				78	75				Diet only
May 4					225				Diet only
May 9			4.344	52.4	150	9.3	9.7	10.8	Diet only
May 13	12.7	22	4.448	53.5	40	9.5	9.9	2.7	Diet only
May 18		24	4.800	52.4	140	6.4	9.7	10.0	Diet only
May 23		25	6.112	48	30	5.3	8.9	2.0	Diet—ferric citrate—0.8 gram
May 30		23	4.180	50.3	30		9.3		Diet—ferric citrate—0.8 gram
June 3				53	110		9.6	8.0	Diet—ferric citrate—0.8 gram
June 9	11.8	26	5.520	54.1			10.0	9.0	Diet—ferric citrate—0.8 gram
June 13			6.432	66.5	150		12.3	13.7	Diet—ferric citrate—0.8 gram
June 20		28		61.6	200		11.4	17.0	Diet--ferric citrate—0.8 gram
June 26			6.864	67.1	20		12.4		Liver 300 grams
July 2				63.3	215		11.7	18.7	Liver 300 grams
July 8				62.2	200		11.5	17.7	Liver 300 grams
July 14				60	20		11.1	2.4	Diet only
July 20				65	100		12.1	9.0	Diet only
July 25				48	30		8.8	2.0	Diet only

PROTOCOLS—*Continued*

Date	Weight	Hematocrit	Red cell counts	Hemoglobin level	Amount bled	Reticulocytes	Oxygen capacity	Hemoglobin	Experimental diet
				Dog No. 72					
1927	*kgm.*	*per cent*	*millions*	*per cent*	*cc.*	*per cent*	*volumes per cent*	*grams*	
March 21	15.5			120	300	1.0	22.3	50	Diet only
March 23				98.5	190		18.2	27	Diet only
March 25				81.1	170		15	19	Diet only
March 28		23	4.064	73	150	4.3	13.5	15	Diet only
April 1		21	5.760	72	100			11	Diet only
April 6		23		76	150			16.5	Diet only
April 8		23	6.922	71	100	3.0	13.2	9.8	Diet only
April 13		24	5.992	74	200	4.2		21.8	Diet only
April 16		26		65	180	4.0	12.0	16.1	Diet only
April 18	14.8		5.720	50	40	4.9		2.6	Diet only
April 22	14.2		5.936	62	60	5.1	11.5	5.2	Diet only
April 25			5.584	56	150	1.0		11.6	Diet only
April 27			5.048	53	30	4.8		2.1	Diet only
April 29			4.768	58.1	20	6.7	10.7	1	Diet
May 4			5.696	50.1	100	4.7	10.0	7.5	Diet
May 10			6.184	51.3	115	5.9	9.5	7.1	Diet only
May 13		21	5.312	45.7	200	7.4	8.45	12.4	Diet only
May 11		19	4.976	39.1	200	6.7	7.24	10.8	Diet only
May 27	14.8		5.392	36	210	6.3		10	Diet only
May 30			5.300	34	30	5.9	6.35	1	Blaud's pills—10 per day
June 6		22	5.010	46	125	3.3	8.5	8	Blaud's pills—10 per day

June 10	15.0			61	160		11.4	13.6	Diet only
June 13			7.184	59	150		10.9	12.2	Diet only
June 20			6.144	42	210		8.3	13.2	Diet only
June 22			6.432	46	30		8.5		Ferric citrate—nucleic acid—20 grams
June 29				57.4	240		9.5	17.0	Ferric citrate—nucleic acid—20 grams
July 8				44.7	100		8.2	6.2	Ferric citrate—nucleic acid—20 grams
July 14			5.688	38	160		7.1	8.5	Ferric citrate—nucleic acid—20 grams
				Dog No. 2					
December 28	11.6	40		102	225			31	Bread and milk
December 30		32		72	240			24.8	Bread and milk
January 4		24		70	200			19.3	Bread and milk
January 6			3.656	50	20				Thymus—400 grams
January 8		23	3.744	53	20				Thymus—400 grams
January 11		24.5	3.184	47	220			14.2	Thymus—400 grams
January 13		20	3.520	57	15				Thymus—400 grams
January 15		22	3.568	62	200			17.1	Thymus—400 grams
January 18	11.0	21.5	3.056	54	25				Bread and milk
January 22		20.5	4.200	56	150			11.6	Bread and milk
January 25		18.5	4.344	56	145			11.2	Bread and milk
				Dog No. 3					
January 20	11.0	38		108	225				Bread and milk
January 22		32		88	250				Bread and milk
January 25		30.4		75	240				Bread and milk
January 27			5.000	64	30				Liver—400 grams
January 29		26.4	5.400	62	60				Liver—400 grams

PROTOCOLS—*Continued*

Date	Weight	Hematocrit	Red cell counts	Hemoglobin level	Amount bled	Reticulocytes	Oxygen capacity	Hemoglobin	Experimental diet
Dog No. 3—Continued									
1927	*kgm.*	*per cent*	*millions*	*per cent*	*cc.*	*per cent*	*volumes per cent*	*grams*	
February 1		23.0	5.184	62	160			13.7	Liver—400 grams
February 5			5.056	56	140			10.8	Liver—400 grams
February 6									Bread and milk
February 10	11.8	20	5.312	50	30				Bread and milk
February 14		24	4.176	60	20				$FeCO_3$—0.6 gram
February 18		25.4	6.248	65	325			29.1	Thymus—400 grams
February 23				58	30			2.4	Thymus—400 grams
February 25									Diet only
February 28		27.5		70	75			7.2	$FeCO_3$—0.6 gram
March 2		26.8		67	240			22.9	$FeCO_3$—0.6 gram
March 4		19	5.272	48	30				$FeCO_3$—0.6 gram
March 9	13.2	23.3	6.744	67	40				$FeCO_3$—0.6 gram
March 14		25.4	6.992	66	220		12.2	20	Diet only
March 16			5.357	50	15	0.7			Diet only
March 21		22	5.960	54	40	2.7		3.0	Diet only
March 35		21	5.944	55.7	75	2.3	10.3	5.8	Diet only
March 30			7.224	62.3	30	3.5	11.5	2.6	Diet only
April 4		24	7.024	52	30	2.3	9.6	2.2	Diet only
April 8		23	7.624	57	100	2.8	10.7	8.0	Diet only
April 11	13.0		5.928	50	15	1.4			Yeast—nucleic acid—15 grams
April 15		23	6.528	51.4	140	4.0	9.5	9.8	Ferric citrate—0.8 gram

April 18	13.4	26	7.008	66	115	3.9		10.5	Ferric citrate—0.8 gram
April 20			6.908	58	90	5.0	10.7	7.1	Diet—ferric citrate
April 25			6.568	63.4	165	4.0	11.7	14.4	Diet—ferric citrate
April 27			7.530	62	100	4.7		8.6	Diet—ferric citrate
April 29			5.072	55	165	6.3		12.6	Diet—ferric citrate
May 4	13.4		6.640	55	30	6.6	10.2		Diet—ferric citrate
May 8			7.856	68.7	125	3.4	12.8	12	Diet—ferric citrate
May 11		33	8.176	64	180	4.9		16	Diet—ferric citrate
May 13	12.7	26	7.120	54	40	5.3	10.0	3.0	Diet—ferric citrate
May 18			7.200	54.6	200	4.3	10.1	15.0	Diet—ferric citrate
May 23		27	6.656	54.6	100	2.9	10.1	7.5	Diet—ferric citrate
May 30			4.650	52.6	150	2.8	9.7	10.8	Diet—ferric citrate
June 6			8.660	66	125	4.0	12.4	11.6	Diet—ferric citrate
June 10				70	30		13.0	2.9	Diet—ferric citrate
June 17			8.568	72	210			21.0	Diet—ferric citrate
June 24				60.6			11.2	20.0	Diet—ferric citrate
					Dog No. 4				
February 1	11.1	36	8.290	100	200				Diet—$FeCO_3$—0.6 gram
February 3			5.750	75	115				Diet—$FeCO_3$—0.6 gram
February 5				68	160				Diet—$FeCO_3$—0.6 gram
February 8		18.4	3.750	52	135				Diet—$FeCO_3$—0.6 gram
February 10		20.0	3.960	50	30			2.0	Nucleic acid—15 grams
February 14		23.0	4.512	64	60			4.3	Nucleic acid—15 grams
February 16				65	75			6.7	Nucleic acid—15 grams
February 18		27.7	5.056	63	190			16.6	Nucleic acid—15 grams
February 21				56	30			5.0	Diet only
February 25	11.8	26.6	5.532	68	90			8.4	$FeCO_3$—0.6 gram

PROTOCOLS—*Concluded*

Date	Weight	Hematocrit	Red cell counts	Hemoglobin level	Amount bled	Reticulocytes	Oxygen capacity	Hemoglobin	Experimental diet
				Dog No. 4—continued					
1927	*kgm.*	*per cent*	*millions*	*per cent*	*cc.*	*per cent*	*volumes per cent*	*grams*	
February 28		24.0		65	100			9.6	$FeCO_3$—0.6 gram
March 2		23.7		61	300			25.2	$FeCO_3$—0.6 gram
March 4		19.7	4.568	52	40				$FeCO_3$—0.6 gram
March 7			4.936	53	35	7.6	7.8	2.6	$FeCO_3$—0.6 gram
March 11	12.2	20.0	4.256	62	170	2.5		14.5	$FeCO_3$—0.6 gram
March 14									Diet only
March 16			4.912	53	40	3.2			Diet only
March 25		20	5.232	47	100	2.7		6.6	Diet only
March 30			4.928	43	40	4.4			Diet only
April 4		21	4.720	38.5	30	1.0			Diet only
April 8		18	5.176	38	25	4.7		1.0	Diet only
April 10	12.2								Diet—ferric citrate
April 13		22	5.360	52.1	30	5.2	8.74	2.0	Diet—ferric citrate
April 18	12.7	27	6.632	64	75	4.8		4.2	Diet—ferric citrate
April 20			6.652	62	150	6.7		12.8	Diet—ferric citrate
April 25			6.992	66.5	150	3.8		13.8	Diet—ferric citrate
April 27			6.182	65	160	3.4		14.0	Diet—ferric citrate
April 29			6.508	56	160	6.7		12.0	Diet—ferric citrate
May 4	12.2	30	5.312	57	30	7.0	10.4		Diet—ferric citrate
May 9			7.184	61.6	100	4.2	11.4	8.5	Diet—ferric citrate
May 11		32	7.584	67	220	4.1		20.0	Diet—ferric citrate

May 18	12.0		6.896	57	200	3.8	10.5	15.7	Diet—ferric citrate
May 23		29	7.152	61	200	5.8	11.3	16.8	Diet—ferric citrate
May 25			6.400	54	30	10.1	10.0	2.0	400 grams liver—ferric citrate
May 30		27	5.280	49.2	150	7.8	9.1	10.2	400 grams liver—ferric citrate
June 3				56.2	125	6.9	10.4	9.7	400 grams liver—ferric citrate
June 5									Diet—ferric citrate
June 8	13.0	32	6.890	60.8	125		11.2	10.5	Diet—ferric citrate
June 13			6.580	64	180		11.8	15.8	Diet—ferric citrate
June 20		30		63.6	210		11.7	18.2	Diet—ferric citrate
June 22			6.128	65	30		12.1		Diet—ferric citrate
July 2				63.8	30		11.8		Diet—ferric citrate

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