

THE RÔLE OF THE SPLEEN IN IRON METABOLISM AS
ELUCIDATED BY CHANGES IN THE IRON BAL-
ANCE AFTER SPLENECTOMY*

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Previous work in this school (Pearce, Krumbhaar, and Frazier) and elsewhere (Asher and Grossenbacher, Asher and Neuenschwander, Asher and Scheinfinkel, Asher and Vogel, and Asher and Zimmermann) on the rôle of the spleen in relation to iron metabolism has yielded discordant results. It therefore seemed worth while to repeat some of the work reported using more modern methods of iron determination, a larger number of animals, and a longer series of controls, both of intact and operated animals, studied over longer periods.

Since the literature of the subject has been adequately reviewed by Pearce, Krumbhaar, and Frazier (1918) and recently in a review of the whole subject of iron metabolism by Lintzel (1931), we shall only summarize it briefly here.

In studies carried out on various occasions over a number of years on rabbits, dogs, guinea pigs, and rats, Asher and his coworkers (1909-28) found a marked and permanent increase of iron elimination after removal of the spleen. Partial compensation for the loss of the splenic storehouse of iron they believed to be demonstrated by the increase in the iron content of the liver after splenectomy. They concluded that the spleen acts to prevent the loss of iron from the body and is therefore the principal organ of iron metabolism. Pearce and his associates in this school (1912-17), in their studies of iron elimination after splenectomy, were unable to demonstrate a constant increase in the iron output. While they found several instances in which there was a significant increase in iron output in a

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† This is the seventeenth in a series of papers on the relation of the spleen to blood destruction and regeneration and to hemolytic jaundice beginning in *J. Exp. Med.*, 1912, **16**, and appearing mostly in that *Journal*.

splenectomized animal, this increase did not occur in every animal and when found was associated with a marked secondary anemia. Anemia of a degree not found in animals subjected to other operative procedures was found by these investigators in splenectomized animals with sufficient frequency to make them feel warranted in associating its occurrence with the removal of the spleen. The exact reason for its appearance, however, they were unable to determine. Asher and Vogel (1912), on the other hand, were unable to demonstrate anemia in splenectomized dogs unless they kept them on an iron-free diet. Later investigators, Lauda and Haam (1927), explained the postsplenectomy anemia in rats as due to the lighting up of infection by *Bartonella muris*, which is customarily held in check by the spleen. Kikuth (1929) has described a similar blood parasite (*Bartonella canis*) which he believes to be a cause of postsplenectomy anemia in dogs. Study of stained spreads of splenectomized dog's blood, however, on many occasions over many years, as well as the differences in the dog's and the rat's anemia, have convinced us that microorganismal cell inclusions play no part in the regularly appearing anemia of the dog.

The effect of artificially induced anemia on iron elimination has been investigated by Asher and Zimmermann (1909) and by Dubin and Pearce (1917-18). The former in poisoning with acetylphenylhydrazine (pyrodine) reported a marked increase in the elimination of iron in the feces in an intact animal (dog), and in a splenectomized animal an increased elimination greatly exceeding that which they had found to occur spontaneously after removal of the spleen. The latter, on the other hand, found no increase in iron elimination in the transient anemias in dogs produced by such agents as sodium oleate (two dogs, studied in periods of 6 to 7 days before administration, and during the anemifying and recovery periods), toluylenediamine (two dogs, periods of 6 and 7 days before and during the production of anemia), and hemolytic serum (two experiments) in the absence of hemoglobinuria; or in a chronic anemia studied in six animals infected with *T. equiperdum*, two of which were splenectomized at the height of the anemia. The iron set free by blood destruction they accounted for by the increase in the iron content of liver and spleen in both the various acute and the chronic forms of anemia which they studied.

Lintzel and Radeff (1930) have suggested that the liver and spleen in combination are the great storehouses of iron in the body, and that the spleen is most active in furnishing available iron through the destruction of erythrocytes. They draw these conclusions from studies of rats on an iron-free diet, exposed to varying degrees of atmospheric pressure. In determinations of the total hemoglobin iron, and of iron in the liver and the spleen under normal atmospheric pressure, after adaptation to a low pressure chamber, and at several stages of reacclimatization to a normal pressure, they found that under all conditions the sum of the three remained a constant, so that as the hemoglobin iron rose the sum of liver and spleen iron fell and *vice versa*. But most significant is their finding that whereas the ratio of spleen iron to liver iron at either of the two extremes of pressure was the same, it was much higher at a point about midway toward readaptation to normal pres-

sure. This they regard as evidence that the decreasing circulatory hemoglobin is disposed of within the spleen.

Present Study

Thirteen dogs were studied, taken from the usual laboratory stock obtained from miscellaneous sources. Complete blood examinations were made in all and simultaneous determinations of iron intake and output (two to eight periods in the various animals).

Six dogs were splenectomized and seven were used to study possible changes in iron balance in intact animals or in those recovering from other major operations, surgically comparable to splenectomy. Thus, the experiment was controlled as follows: (1) Observations in the preoperative period served as an individual control for each of the animals later operated upon; (2) two intact animals were followed (one for more than a year) to evaluate variations in intake-output relationships that might otherwise be considered abnormal; (3) in order to exclude the effects of a major operation *per se*, two dogs were studied before and after nephrectomy, two before and after resection of one Fallopian tube, and one before and after a partial omentectomy. All operations were performed under a general anesthetic (amytal-ether or ether), with the usual aseptic technique.

The whole question of the iron balance in the normal animal will not be satisfactorily understood until the factors determining iron storage have been better explained. That storage of iron may be influenced by the iron intake in a growing animal has recently been suggested by the work of Leichsenring and Flor (1932) on young children. They found that on a high iron intake more iron is stored than when the intake is low. It would seem, therefore, that in considering the iron balance no hard and fast conclusions can be drawn from mere comparison of one balance with another, as has been customary.

Methods

1. *Diet.*—In order to have a uniform ration we have used the Cowgill-Karr synthetic diet. This diet contains casein, sucrose, lard, and cod liver oil, with supplementary salts, vitavose (for vitamin B), and bone ash for roughage. In one instance Valentine's extract-free dried meat was substituted for casein (Dog 1, first postoperative period). The Cowgill diet is designed to furnish 80 calories and 0.8 gm. of protein nitrogen per kilo unit. We have varied the iron content of the diet by altering the amount of ferric citrate in the salt mixture, the range being from 6 to 15 mg. of total iron per 100 gm. of ration. The ration was made up in batches of 2 kilos and was kept covered in glass jars at low temperature in an electrical refrigerator. Frequent analyses for iron were made. 16 gm. of food mixture per kilo of body weight constituted the daily feeding. The animals were weighed and fed at the same time each day and a record kept of weight, food given, and food consumed from which the daily iron intake was calculated.

This ration was not always eaten with good appetite. The reason for this we

were not able to determine. Our experience has been that while a dog might eat all that was offered it for a time, appetite frequently failed after a week or so on the synthetic diet. In cases of failure of appetite no marked improvement has been noted when more vitamin B (as vitavose, Squibb) has been added to the feedings.

2. *Collection of Feces.*—The dogs were kept in metabolism cages with glass floors. After several days on the synthetic diet, carmine or charcoal was given by mouth to mark the beginning of a collection period. The periods of collection varied in length from 6 to 10, and in several instances, 14 days. At the end of the period, charcoal or carmine was again given. The feces were kept in covered glass jars in a hot-air oven until ready for analysis.

3. *Analysis of Feces for Iron.*—The dried feces were ground to a powder in a mortar, weighed, and aliquot portions taken for analysis.

Walker's modification of the Thomson method was used in the analysis of the feces for iron, with a further modification that we adopted from the wet-ashing method of Kennedy. A method practically similar to the one we used was later described by Elvehjem.

The sample was ashed in a porcelain crucible in an electric muffler furnace. The ash was dissolved in 5 cc. of concentrated nitric acid to which was added 10 cc. of water and 0.5 cc. hydrogen peroxide. The dissolved ash was transferred by washing to a volumetric flask and made to volume. A standard solution was made by diluting a stock standard solution, containing 0.1 mg. of iron per cent, to a known volume (50 to 100 cc.) with 2 cc. of concentrated nitric acid and a few drops of hydrogen peroxide. The stock standard solution contained 0.7 gm. ferrous ammonium sulfate in 0.5 per cent sulfuric acid with sufficient N/5 potassium permanganate for complete oxidation (Thomson).

To 10.0 cc. of the diluted standard solution in a 50.0 cc. volumetric flask, were added 5.0 cc. of 20 per cent potassium thiocyanate solution and 10.0 cc. of amyl alcohol. After shaking, the amyl alcohol layer, containing the ferric thiocyanate, was pipetted into the cup of a colorimeter.

Sufficient of the unknown solution was measured into 5 cc. of 20 per cent potassium thiocyanate to develop a color approximating that of the standard. 10 cc. of amyl alcohol were added and the whole shaken. The amyl alcohol layer was pipetted off and the two solutions compared in the colorimeter. The percentage iron content of the sample of feces was calculated according to the equation $S/U \times D \times 100/A = \text{mg. iron in sample of feces, when}$

S = reading of the standard

U = reading of the unknown

D = dilution factor

A = weight of the sample

Analysis of Results

In analyzing our results we have taken as the indicator of the iron metabolism the iron balance; *i.e.*, the difference between output and

intake of iron in milligrams per kilo of body weight per day. A positive iron balance thus indicates an output less than intake, a negative or deficit iron balance the reverse. Loss of iron in hair, dandruff, urine, etc., has not been taken into account, being so slight that it would not materially affect results, but theoretically at least this omission should tend toward determination of a slightly positive balance in animals in iron equilibrium.

Results in Control Animals.—1. The animals later splenectomized, upon which preoperative observations were made, will be discussed later in comparing directly preoperative with postoperative results.

TABLE I
Iron Metabolism of Intact Dogs

Dog No.	Period studied	Weight	Intake iron*	Balance of iron*
	1929	kg.		
7	6/ 3- 6/13	12.65	2.40	+0.904
	6/19- 6/29	13.33	2.40	+0.585
	7/ 7- 7/15	12.51	0	-0.368 (fasting)
	9/15- 9/25	15.41	0.75	+0.100
	10/27-11/6	13.19	0.29	-0.145 (losing weight)
	1930			
	1/ 3- 1/10	13.39	1.12	+0.442
8	6/23- 6/30	6.9	1.09	+0.582
	7/13- 7/20	7.0	2.26	+1.263

* Figures represent milligrams iron per kilo per day.

Suffice it to say here that before splenectomy the iron balances varied considerably for reasons that were not always ascertainable and that both positive and negative balances were observed. Variation of balance in relation to intake and to change in weight is also discussed later.

2. The intact control (Table I) upon which we have made observations for over a year and a half has also shown variation in the iron balance. It was in positive balance (0.4 mg. or over) throughout the time studied except when fasting or when the total food intake was much reduced, iron intake being correspondingly reduced to a very low level. In this latter period the dog lost over 1 kilo in weight

and had a negative balance of 0.145 mg. per kilo per day. The second dog which was studied 2 out of 5 weeks also had a positive balance in

TABLE II

Iron Metabolism of Dogs before and after Major Operations Other than Splenectomy

Dog No.	Operation	Period studied	Weight	Intake iron*	Balance of iron*	Blood picture, etc.
9	Nephrectomy 2/12 (ether)	1930 1/20-1/27	kg. 14.86	1.136	0.423	Unchanged
		1/27-2/2	15.06	0.972	0.103	
		3/ 3-3/17	14.75	0.579	0.351	
		3/20-4/3	14.95	0.633	0.331	
		10/20-1/27	12.57	0.583	0.188	
10	Nephrectomy 1/29 (amytal- ether)	2/ 8-2/14	11.66	0.936	0.057	Beginning anemia
		2/14-2/20	11.73	0.503	0.094	Recovery from anemia
		1931 2/13-2/20	13.24	0.852	0.075	
11	Partial omentec- tomy 3/25/31 (amytal)	4/ 1-4/7	12.71	0.788	-0.022	Postoperative anemia
		1930 12/30-1/6	9.67	1.824	0.309	
12	Unilateral salpin- gectomy 1/7/31 (amytal-ether)	1931 1/13-1/20	9.83	1.146	-0.181	Anemia
		1931 6/23-6/30	5.8	1.192	0.304	
13	Right salpingec- tomy 7/9 (amytal)	7/24-7/31	5.6	2.314	0.989	No anemia

* Figures represent milligrams iron per kilo per day.

both, and showed a marked increase in the balance at the high level of intake.

3. Five dogs were subjected to major operations other than splenectomy (Table II), two of which developed a mild grade of anemia. Before operation all of this group had positive iron balances ranging from 0.423 to 0.075 mg. per kilo per day. After operation positive balances ranging from 0.989 mg. to 0.057 mg. per kilo per day were found in three of the animals. One, after partial omentectomy had a very small negative balance, 0.022 mg., and a second animal after unilateral salpingectomy had a larger negative balance, 0.181. Both of these animals developed a mild postoperative anemia and one in addition had an abscess in the subcutaneous tissue of the abdomen.

4. *Results in Splenectomized Animals (Table III).*—Dog 2 before operation, in three periods, had first a negative balance on a very low intake, and then positive balances, increasing with added intake. It is interesting to note that this animal, although unable to maintain weight, stored iron increasingly as the intake of iron increased. Recovery from operation was stormy, and collections could not be begun until 18 days after operation. At that time the animal had not only developed an anemia, but was on the road to recovery—the reticulocytes during the first period in which collection of feces could be made rising to 3.6 per cent. Intake was much increased and storage was considerable. In the following week, intake and storage were slightly less. In a period 4 months after operation, the balance was very slightly negative.

Dog 1 before operation at first stored iron, but after a fasting period had a very low intake of iron and a noticeably negative iron balance. After operation this animal made a very poor recovery, was listless, did not eat, and had periods of vomiting and diarrhea. In the first 2 weeks she developed an anemia (erythrocytes fell 1.3 millions and hemoglobin 24 per cent), but because of the diarrhea no collection of feces could be made. In the first period, 1 month after operation and in the second, 7 weeks after operation, it is important to note that she still was in as great negative iron balance as before operation, although the intake of iron was greater than in the periods of negative balance before operation. 5 months after splenectomy there was again a negative balance during a period of secondary anemia.

Dog 3 was in positive balance before operation and for two periods studied up to 24 days after operation. The balance was slightly less

TABLE III
Iron Balance before and after Splenectomy

Dog No.	Period studied	Weight kg.	Intake iron*	Balance of iron*	Erythrocytes millions per c.mm.	Hemoglobin (Sahlb) per cent	Reticulocytes per 100 erythrocytes	Time after splenectomy	
2	1929 12/ 2-12/9 12/10-12/17 12/ 8-12/25	11.55	0.217	-0.045	7.35	110	0.1		
		10.67	0.318	+0.020	7.98	124	0.1		
		10.25	0.400	+0.103	7.68	132	—		
		12/30/29 splenectomy (amytal-ether)							
	1930 1/17- 1/24 1/24- 1/31 5/13- 5/20	8.78	1.151	+0.565	4.63	92	3.6	18 days	
		9.157	1.134	+0.295	6.02	94	1.7	25 days	
		13.64	0.639	-0.035	7.8	98	0.24	4 mos.	
1	1929 10/30-11/7 11/19-11/26 12/ 3-12/10	14.01	0.957	+0.349	6.65-6.45	97	0.68-0.44	Fasting	
		12.258	0	-0.253	—	—	—		
		11.72	0.125	-0.129	7.6	110	—		
		12/10/29 splenectomy (amytal-ether)							
	1930 1/10- 1/20 1/27- 2/2 5/13- 5/20	10.25	0.360	-0.097	6.07-6.61	90	0.7-0.1	1 mo.	
		9.885	0.797	-0.242	6.78	90	—	7 wks.	
		13.76	0.518	-0.107	5.69	93	0.4	5 mos.	
	3	1929 11/20-11/27 12/ 3-12/10	10.31	0.998	+0.778	7.6	103	0.1	
			8.83	0.220	+0.1283	7.9	110	—	
		12/11/29 splenectomy (amytal)							
8.17		1.173	+0.107	7.65	126-103	0.05	6 days		
8.56		1.10	+0.140	7.17	110	0.18	17 days		

4	¹⁹²⁹ 4/10-4/21	8.641	1.25	-0.135	6.94	105	—	—
		4/30/29 splenectomy (ether)						
	5/ 2- 5/16	8.14	1.437	-0.57	7.56-6.21	92-110-96	0.67-0.18	2-16 days
	6/ 8- 6/18	8.37	1.86	-0.48	5.35	83	1.0	39-49 days
5	¹⁹³⁰ 4/25- 5/2	8.11	1.213	+0.131	5.63	79	0.35	1 yr.
	¹⁹²⁹ 4/10-4/21	12.73	0.65	-0.046	6.2	95.5	—	—
		4/30/29 splenectomy (ether)						
6	5/ 1- 5/12	12.84	1.59	+0.304	7.45-6.15	94-92	1.4-0.1	1 day
	6/ 8- 6/18	13.67	1.803	-0.90	6.07-5.67	89-85	4.5	39 days
	7/ 7- 7/15	12.01	0	-0.590	5.72	92	0.1	(Fasting) 2 mos.
	¹⁹³⁰ 4/25- 5/2	13.16	1.08	+0.473	6.34	97	—	1 yr.
	9/15- 9/25	12.2	0.579	+0.001	—	—	—	18 mos.
	11/ 5-11/15	12.39	0.919	+0.587	6.485	94	0.3	18½ mos.
	¹⁹²⁹ 6/ 4- 6/12	9.6	1.54	+0.279	6.9	100	0.1	—
		6/13/29 splenectomy (amylal-ether)						
	6/17- 6/27	8.88	0.908	+0.305	6.56-4.9 (6/29)	96-80	1.3-3.6	4-14 days
	7/ 6- 7/16	8.54	1.49	-0.083	5.27-5.57	79-91	0.1	24 days
	10/15-10/22	8.8	1.085	-0.157	7.6	98	0.1	4 mos.
	10/23-10/30	8.48	0	-0.322	6.42	90	—	(Fasting) 4 mos.
¹⁹³⁰ 5/13- 5/20	8.8	2.077	+0.517	6.48	104	0.61	11 mos.	
9/15- 9/25	9.95	0.977	+0.1	—	—	—	15 mos.	
10/27-11/6	9.08	1.22	+0.288	6.43	91	0.05	17 mos.	

* Figures represent milligrams iron per kilo per day.

but still positive for 24 days after operation on an iron intake that exceeded the preoperative intake. This dog developed later a post-splenectomy anemia, but the iron balance unfortunately could not be determined at that time.

Dog 4 had a small negative balance before operation, which increased during the 6 weeks following splenectomy when an anemia developed. 1 year after splenectomy the animal was in positive balance.

Dog 5 was in negative balance before splenectomy. In the 11 days immediately after the operation there was a positive iron balance. During this period there was a rise of the erythrocyte count, hemoglobin, and reticulocytes, of 2 days' duration, and a subsequent slow fall of all these. 1 month after operation there was a frank secondary anemia, and in a 10 day period at this time there was a distinct negative balance, with less anemia and a slightly negative balance at 2 months. In periods 1 year, 17 months, and 18½ months after splenectomy there was a positive balance with the blood count at the preoperative level.

Dog 6 had a positive balance before operation and a still larger balance in the first 2 weeks after operation. Toward the end of the 1st month, when the postsplenectomy anemia was at its height, there was a slight negative balance. In periods 11, 15, and 17 months after splenectomy the balance was positive.

We have found, therefore, in five of the six dogs splenectomized (all but No. 2) a greater tendency to loss of iron after removal of the spleen, which is seen as either a change from a positive to a negative balance, or to an increasingly negative or a lessened positive balance. (In Dog 3, which fell in the last category, no iron studies were made during the height of the anemia.) This phenomenon is transient and usually coexistent with a period of postsplenectomy anemia which we have found in all the dogs we have so far studied.

Further consideration shows the close correlation between the blood picture and the iron balance following splenectomy. It will be noted (Table III) that if the reticulocyte count rose immediately after splenectomy and hemoglobin and erythrocytes either remained near or rose slightly above the preoperative level, there was a positive iron balance (Dogs 4, 3, and 6). When the fall of the erythrocytes and

TABLE IV
The Iron Balance in Relation to Iron Intake and to Change in Weight

Intake	Iron balance*				Change in weight kg.
	Intact	After splenectomy		After other major operations	
		1-2 mos.	Over 2 mos.		
2.404	+0.904				+0.5
2.400	+0.585				+0.3
2.264	+1.263				±0.0
2.077			+0.517		+0.6
2.314				+0.989	±0.0
1.86		-0.48			-0.2
1.80		-0.09			±0.0
1.59		+0.304			+0.4
1.54	+0.279				±0.0
1.44		-0.57			-0.95
1.49		-0.083			+0.5
1.213			+0.131		-0.5
1.22			+0.288		—
1.17		+0.107			+0.2
1.14	+0.423				+0.1
1.15		+0.365			+0.4
1.13		+0.295			+0.4
1.12	+0.442				±0.0
1.085			-0.157		—
1.08			+0.473		+0.2
1.09	+0.588				+0.2
1.095		+0.140			+0.1
1.192		+0.504			+0.2
1.146				-0.181	+0.2
0.998	+0.778				±0.0
0.972	+0.103				±0.0
0.936				+0.057	-0.2
0.908		+0.305			-0.5
0.957	+0.349				+0.1
0.977			+0.1		—
0.919			+0.587		±0.0
0.852	+0.075				+0.3
0.788				-0.022	+0.2
0.797		-0.242			-0.2
0.650	-0.05				-0.4
0.639			-0.035		-0.3
0.579			+0.001		—
0.633				+0.331	+0.25

TABLE IV—*Concluded*

Intake	Iron balance*				Change in weight <i>kg.</i>
	Intact	After splenectomy		After other major operations	
		1-2 mos.	Over 2 mos.		
0.570				+0.351	+0.2
0.518			-0.107		-0.4
0.400	+0.103				0.0
0.360		-0.097			-0.4
0.318	+0.02				-0.3
0.297	-0.145				-0.1
0.220	+0.128				-0.2
0.217	-0.045				-0.6
0.125	-0.129				-0.2

* Figures represent milligrams iron per kilo per day.

TABLE V
Weight Change in Relation to Iron Balance

		Intact	After splenectomy		After other major operations	Total
			Early	Late		
Gain in weight	positive balance....	6	6	2	2	16
	negative balance....	0	1	0	2	3
Stationary weight	positive balance....	6	0	1	1	8
	negative balance....	0	1	0	0	1
Loss of weight	positive balance....	2	0	2	1	5
	negative balance....	5	4	2	0	11

hemoglobin occurred, the balance tended to become negative (Dogs 4, 5, 6, 1) unless the rise in the reticulocytes was rapid (Dogs 3, 2).

In Table IV we have charted the iron balance in relation to the iron intake. The figures for intake are necessarily in close relationship to the total food intake, so that an adequate iron intake indicates adequate intake of protein and of total calories, and, similarly, decreasing iron intake indicates a reduction of protein and of calories. It will be noted that while there is variation in the amount of iron retained, there is a tendency toward a positive balance in intact dogs, considered

in order of decreasing iron intake, until the level of about 0.7 mg. of iron per kilo per day is reached. Below that level four of seven intact dogs were in negative balance. The early splenectomized animals, on the other hand, show at all levels of intake a tendency toward a negative balance within the first 2 months after removal of the spleen. In all cases these negative balances are correlated with periods of anemia. In periods after 2 months there are but two instances of negative balance in the splenectomized group. It will be noted, however, that the iron intake in splenectomized animals was in no instance below 0.5 mg. per kilo per day. After major operations other than splenectomy (one in a dog having an intake of over 1.0 mg. of iron per kilo daily, the other of 0.787 mg.), there are two instances of negative balance in both of which there occurred at the same time postoperative anemia.

While gain or loss of weight may presumably be associated with the amount of the intake, we have thought it of interest to tabulate our findings of change of weight in relation to the iron balance. These findings are summarized in Table V. Gain in weight was associated preponderantly with a positive balance. In the three instances in which gain was associated with a negative balance, anemia occurred, in one animal after splenectomy and in the other two after other operations. Positive balances were constantly found in intact animals associated with gain in weight. Positive balances were also found where the weight was maintained without change, except in one instance. In this the animal had a severe postsplenectomy anemia. Loss of weight, on the other hand, was not associated with a negative balance to the same extent as was gain of weight with a positive balance, five of the sixteen instances of actual loss of weight during a given period being associated with positive balances. Two of these were in intact animals, both of which were in the low-intake group. One was in a recently splenectomized animal (No. 2) with a fairly high reticulocyte count, and another was in an animal splenectomized 1 year before, which had a substantial iron intake, but lost weight during the period for a reason unknown to us. The fifth was in an animal which had been nephrectomized 10 days before, had a fairly high iron intake and a steadily rising reticulocyte count.

SUMMARY

We have estimated the iron balance in six dogs before and after splenectomy, in two intact dogs, and in five dogs before and after major operations other than splenectomy.

In all the animals studied, considerable variation in iron balance was observed in the periods used (6 to 14 days). The intact controls had positive balances, the one followed for a year and a half having over 0.4 mg. per kilo per day, except when fasting or on an inadequate intake. The iron of urine, lost hair, dandruff, etc., which was not included in the determinations, would be far from sufficient to restore equilibrium.

Of five animals subjected to major operations other than splenectomy, all of which in preoperative periods had positive balances varying between 0.423 mg. to 0.075 mg. per kilo per day, three showed a positive balance of from 0.989 mg. to 0.057 mg. and two showed negative balances of from 0.02 to 0.18 mg., the last two having anemia in the period of study while the others had none.

Of the six splenectomized dogs, five showed a greater tendency to loss of iron after splenectomy (*i.e.*, either a change from a positive to a negative balance or to an increasingly negative or lessened positive balance). This was not always apparent until some days after splenectomy and coincided approximately with the period of developing anemia. The removal of the spleen was usually found therefore to be transiently associated with increased excretion of iron. It is not possible to demonstrate, however, that the increased loss of iron is the cause of the anemia, and the matter awaits further investigation before the reason of its occurrence is made clear.

An adequate iron intake in an intact animal was usually accompanied by a positive balance, though in splenectomized animals during the period of anemia it was frequently accompanied by a negative balance. Gain in weight in intact animals likewise was associated with a positive balance; but in the three instances in which gain of weight occurred during periods of anemia, after splenectomy or other operations, it was associated with a negative balance. Weight maintenance was also associated with a positive balance except in one instance of severe postsplenectomy anemia. Loss of weight was not

sufficiently associated with a negative balance to make it seem a definite factor influencing the balance, *per se*, although about one-third of the periods of loss of weight were associated with negative balances.

CONCLUSIONS

In normal dogs on a diet adequate in iron, removal of the spleen brings about a temporary, increased loss of iron from the body, which is most marked during the period of developing anemia.

It is possible that the postsplenectomy anemia is the cause of the negative balance, though more probable that splenectomy removes an important iron depot and that the loss of iron thus occasioned is the cause of the anemia. Further investigation of postsplenectomy anemia is needed, however, before final conclusions can be drawn as to the mode of its production.

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